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Determination of priority substances and contaminants of emerging concern in drinking water and their removal by chemical processes

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Abstract

The presence of micropollutants in the environment, usually found at trace concentrations (ng L^{-1} to µg L^{-1}), has been highlighted in the last decades. These residual compounds, some of them so-called contaminants of emerging concern (CECs), are not completely removed during wastewater treatment. Therefore, CECs are discharged into receiving water bodies which might be sources for drinking water (DW) supply, bringing several implications for public health. In this context, it is important to set up fast, sensitive and reliable analytical methods that enable the determination of a wide range of pollutants in DW at low concentration levels.

This work describes the optimization and development of a green analytical method based on solid phase extraction (SPE) followed by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), for the fast and simultaneous determination of twenty three pollutants in DW: seven pesticides, one industrial compound, fourteen pharmaceuticals and a metabolite. Some of them were defined as priority substances in the Directive 2013/39/EU or included in the recent watch list of European Commission Decision 2015/495/EU.

UHPLC and MS parameters, as well as several conditions of the SPE procedure were fully optimized. A Kinetex™ 1.7 µm XB-C18 100 Å column (100 × 2.1 mm, i.d.) was used and the optimized mobile phase consisted in ethanol/water (70:30, v/v) at a flow rate of 0.20 mL min^{-1}. The higher recoveries for most compounds were achieved with Oasis® HLB, ethanol as solvent, 250 mL of water samples at pH 3, adding sodium thiosulfate as dechlorinating agent. The SPE-UHPC-MS/MS method was validated, achieving linearity ($R^2 > 0.99$), selectivity and sensitivity within the range of 0.75−40 ng L^{-1} for all compounds. The method detection limits were between 0.01 and 0.02 ng L^{-1} and the method quantification limits were between 0.04 and 0.60 ng L^{-1}. The identity of the compounds was confirmed by the retention time and using two MS/MS transitions and its ion ratios, according to European Commission Decision 2002/657/EC.

The validated method was applied to DW samples from different sources and locations of the north of Portugal, assessing the concentration of the target micropollutants. Thirteen compounds were detected in the DW samples. In addition, lab-scale photolysis (UV) and ozonation experiments were performed for tap water samples, collected from the water supply network of Porto (Portugal) and spiked with the target micropollutants. Only eight pharmaceuticals were completely removed by these water treatments: (i) tramadol, venlafaxine, atorvastatin and azithromycin by both processes; (ii) clopidrogel,

carbamazepine and isoproturon by ozonation; and (iii) the metabolite norfluoxetine by UV photolysis. Regarding to the other substances, the efficiency of the tested processes varied according to the target micropollutant. In this context, other technologies should be applied for the effective treatment of drinking waters.

Keywords: Directive 2013/39/EU; Commission Decision 2015/495/EU; micropollutants; drinking water; solid phase extraction; ultra-high-performance liquid chromatography-tandem mass spectroscopy; ozonation; photolysis.

Resumo

A presença de micropoluentes no ambiente, geralmente detetados em concentrações vestigiais (ng L^{-1} a µg L^{-1}), tem sido alvo de grande destaque nas últimas décadas. Estes compostos, alguns deles designados como contaminantes de preocupação emergente, não são completamente removidos nas estações de tratamento de águas residuais (ETAR) urbanas, sendo posteriormente descarregados nos cursos de água que servirão de fonte de extração de água para consumo humano. A ocorrência de micropoluentes na água potável acarreta diversas implicações para a saúde pública. Neste âmbito, o desenvolvimento de métodos de análise rápidos, de confiança e com elevada sensibilidade que permitam detetar uma vasta gama de micropoluentes em água para consumo é essencial.

O presente trabalho descreve a otimização e o desenvolvimento de um método analítico ecológico, baseado na extração em fase sólida (SPE) seguida de cromatografia líquida de ultra-alta eficiência (UHPLC) associada à espectrometria de massa (MS) em tandem, para a determinação rápida e simultânea de vinte e três poluentes em água para consumo: sete pesticidas, um composto industrial, catorze fármacos e um metabolito. Alguns destes compostos encontram-se definidos como substâncias prioritárias na Diretiva Europeia 2013/39/UE, ou estão incluídos na recente lista de vigilância da Decisão da Comissão Europeia 2015/495/UE.

Os parâmetros do sistema de UHPLC-MS/MS, bem como várias condições do procedimento de SPE, foram completamente otimizados. A coluna usada foi uma Kinetex™ 1,7 µm XB-C18 100 Å (100 × 2,1 mm, d.i.) e a fase móvel otimizada consistiu em etanol/água (70:30, v/v) com um caudal de 0,20 mL min^{-1}. As recuperações mais elevadas foram obtidas com a utilização de cartuchos Oasis® HLB, etanol como solvente, amostras de 250 mL acidificadas a pH 3, adicionando tiossulfato de sódio como agente de remoção de cloro. O método SPE-UHPLC-MS/MS foi validado, conseguindo-se obter linearidade (R^2> 0,99), seletividade e sensibilidade na gama de 0,75—40 ng L^{-1}, para todos os compostos. Os limites de deteção do método situaram-se entre 0,01 e 0,02 ng L^{-1} e os limites de quantificação encontraram-se entre 0,04 e 0,60 ng L^{-1}. A identidade dos compostos foi confirmada através do tempo de retenção e usando duas transições MS/MS e as respetivas proporções entre as fragmentações, de acordo com a Decisão da Comissão Europeia 2002/657/CE.

O método otimizado e validado foi aplicado a amostras de água para consumo, de diferentes origens (rede de abastecimento, fontes e poços) e zonas do norte de Portugal, tendo sido detetados treze micropoluentes. Foram ainda realizados ensaios de fotólise (UV) e ozonização, à escala laboratorial, com amostras de água recolhidas da rede de

abastecimento do Porto (Portugal), posteriormente, contaminadas com os micropoluentes em causa. Apenas oito compostos farmacêuticos foram completamente removidos nos ensaios realizados: (i) tramadol, venlafaxina, atrovastatina e azitromicina pelos dois processos de tratamento; (ii) clopidrogel, carbamazepina e isoproturon por ozonização; e (iii) o metabolito norfluoxetina, quando utilizada a fotólise. Relativamente aos outros compostos, a eficiência dos processos testados varia de acordo com o micropoluente alvo. Neste contexto, outras tecnologias deverão ser aplicadas para o tratamento efetivo de águas para consumo.

Palavras-chave: Diretiva 2013/39/UE; Decisão da Comissão Europeia 2015/495/UE; micropoluentes; água potável; extração em fase sólida; cromatografia líquida de ultra-alta eficiência; espectrometria de massa em tandem; ozonização; fotólise.

Nomenclature

AOP - Advanced oxidation process

CEC - Contaminant of emerging concern

CID - Collision induced dissociation

DW – Drinking water

DWD - Drinking water directive

DWTP – Drinking water treatment plant

EC – European Commission

EDC – Endocrine disrupting compound

EDTA – Ethylenediaminetetraacetic acid

EE2 – Ethinylestradiol

EQS – Environmental quality standards

EU – European Union

GC – Gas chromatography

HLB – Hydrophilic–Lipophilic–Balanced

IDL – Instrument detection limit

IQL – Instrument quantification limit

LC – Liquid chromatography

LLE – Liquid–liquid extraction

LP – Low pressure

MAX – Mixed–mode anion eXchange

MCX – Mixed–mode cation eXchange

MDL – Method detection limit

ME – Matrix effect

MQL – Method quantification limit

MS – Mass spectrometry

MS/MS – Tandem mass spectrometry

MP – Medium pressure

Mw – Molecular weight

MVP – Mercury–vapour lamp

PPCPs – Pharmaceuticals and personal care products

POP – Persistent organic pollutant

PS – Priority substance

PTFE – Polytetrafluoroethylene

QC – Quality control

QqQ – Triple quadrupole

RSD – Relative standard deviation

SPE – Solid phase extraction

SPME – Solid phase microextraction

SRM – Selected reaction monitoring

UHPLC – Ultra–high-performance liquid chromatography

UV – Ultraviolet

UWWD – Urban waste water directive

WFD – Water framework directive

WWTP – Waste water treatment plant

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1. Introduction

1.1. Overview of the problem

In this century, one of the major problems that humanity faces concerns to the quantity and/or quality of water. Although most of the Earth planet is covered by water, only a small amount (ca. 3%) is available as fresh water. The remaining 97% of the total water is present as salty water in oceans, not appropriate for direct drinking, irrigation and most industrial uses (*Figure 1*). According to the European Commission, less than 1% of the Earth's water is available for human consumption and more than 1.2 billion people in the world have no access to safe drinking water [1].

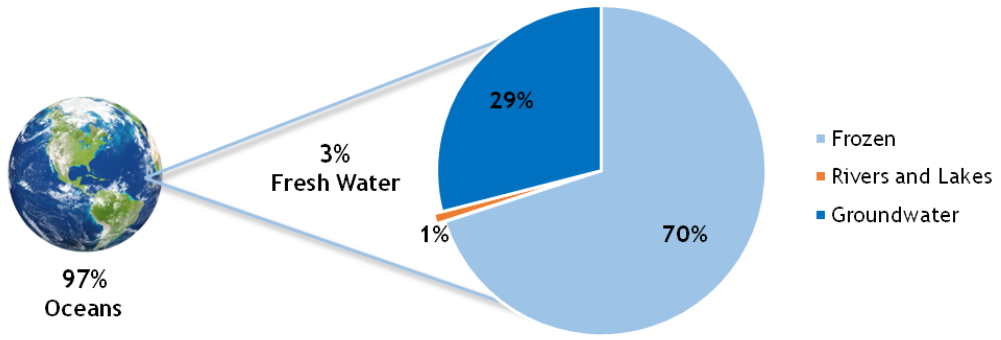


Figure 1 - Distribution of water on earth (Adapted from ref. [2])

Water can be considered a value-added resource since it is crucial to all living organisms and for multiple human activities, such as domestic uses, agriculture and industry. Until the industrial revolution, water demand accompanied the demographic growth, which was slow at that time. However, the following exponential growth of population and the related intensification of the agricultural and industrial activities led to a continuous increase in the demand for fresh water, which tends to be scarce. Besides the growing consumption, the referred factors result also in a great production of wastewater, which direct disposal in the Nature affects negatively the ecosystems. The improvement of life quality and the sustainable development of the World depend on measures to meet the environmental protection and the correction of water problems.

Throughout the past three decades, the research on the impact of water pollution has been centered practically on conventional pollutants, i.e. heavy metals and persistent organic pollutants (POPs), and this issue was extensively reviewed [3]. Nowadays the reduction of emissions in the developed countries, through the launch of policy guidelines to punish the illegal uses and/or discharges [4], took this type of substances from the priority context. In recent years, a growing interest was raised about the fate and effects

of a large group of micropollutants in the aquatic environment, some of them integrated in the so-called contaminants of emerging concern (CECs). These pollutants are found at trace or ultra-trace concentrations (ng L^{-1} to µg L^{-1}) and include pesticides, industrial compounds, pharmaceuticals, personal care products, steroid hormones, drugs of abuse and others [5]. One of the interesting characteristics of these compounds is that they are considered "pseudo-persistent", since their transformation/removal rates are overcome by their continuous introduction into the environment. Moreover, their recalcitrant character together with their polarity, favors their dispersion and interchange between the aquatic compartments.

Several studies have shown that some micropollutants are not completely removed during wastewater treatment, being in this way, discharged into receiving water bodies (rivers, lakes and seas), which may be used as sources for the drinking water (DW) supply [6]. The occurrence of such compounds in DWs brings several adverse implications for public health.

Recently, on 12 August 2013, the European Parliament and of the Council proposed a new Directive 39/2013, amending the Directives 2000/60/EC and 2008/105/EC, where (a) new priority substances (PSs) and groups of PSs in the field of water policy were identified; (b) a first watch list of substances for Union-wide monitoring in the field of water policy was proposed; (c) and the water framework policy updated, highlighting the need to monitor these substances and to develop new water treatment technologies [7]. The first watch list with a provisional character was published in less than two years by the Directive 495/2015 [8], as recommended in the Directive 39/2013.

1.2. Priority substances and contaminants of emerging concern

1.2.1. Definition

According to Article 16 of the Water Framework Directive (WFD) 2000/60/EC, PSs are "individual pollutants or groups of pollutants presenting a significant risk to or via the aquatic environment, including such risks to waters used for the abstraction of drinking water" [9]. Some of these pollutants were already recognized as emerging compounds. The word *emerging* means that these substances are still unregulated (or in process of regulation), have been recently found in the environment and potentially cause harmful effects in aquatic life at environmental concentrations [10]. A contaminant can also be defined "emerging" because of the discovery of a new source, a new pathway to organisms or a more sensitive detection method [10]. These kind of pollutants are usually referred as

CECs due to the unknown risk to the environment and to the human health related to their presence, frequency of occurrence, or source [11].

There are several classes of CECs, such as pesticides, industrial compounds, pharmaceuticals and personal care products (PPCPs), steroids and hormones, surfactants, flame retardants, disinfection by-products, etc. [12].

1.2.2. Pollution sources and environmental fate

Micropollutants are released into the environment through different sources. *Figure 2* shows the possible pathways of these contaminants in the environment.

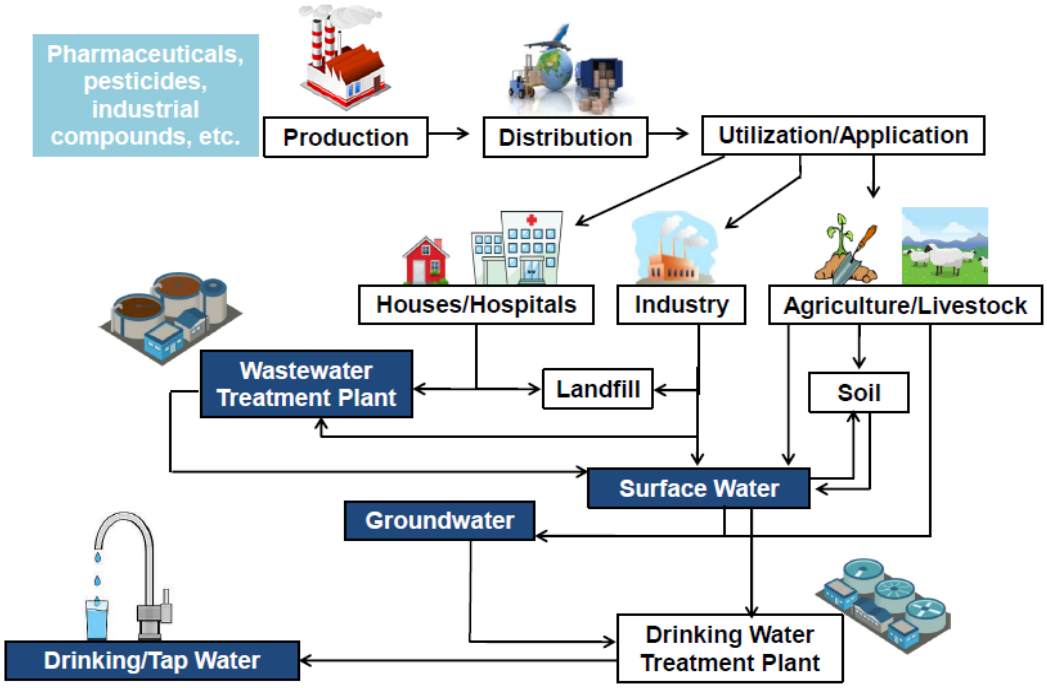


Figure 2 - Representative sources and routes of micropollutants in the environment.

Large amounts of contaminants can be discharged into the aquatic environment after being used, which will further enable their detection in wastewater, surface water, groundwater and even DW, from ng L^{-1} level (or lower) to several µg L^{-1} [13]. Recent studies have demonstrated the occurrence of micropollutants in these matrices [5, 14-16]. These organic substances are often persistent and/or bioaccumulable in the environment, namely in the aquatic compartment.

According to *Figure 2*, the main origin of environmental contamination by CECs is anthropogenic [17]. Industry is one of the sources of contaminants, being CECs either the principal product or used as raw materials to generate new products [18]. The synthetic compounds, such as pesticides, cosmetics, PPCPs, pharmaceuticals and others, are

worldwide used and considered indispensable for modern society [19]. It was shown that the global production of anthropogenic chemicals increased from 1 million to 400 millions tons per each year, between 1930 and 2000 [20]. Statistics published by EUROSTAT in 2013, reveal that over 50% of the total production of chemicals, between 2002 and 2011, is represented by environmentally harmful compounds. More than 70% of these substances are chemical compounds with significant environmental impact [21]. Nevertheless, the effluents produced during manufacturing are negligible due to the good manufacturing practice regulations, required in most countries for the production of pharmaceuticals, personal care products, pesticides and other compounds with environmental impact. Indeed, such emissions are assumed to be low in Europe.

The runoff from the agriculture and livestock areas is another important source of micropollutants, particularly for pesticides used to improve productivity, for steroids hormones and antibiotics used for livestock [22, 23]. In addition, fields irrigated with treated wastewater can also contribute to introduce a lot of contaminants and their intermediates into the receiving waters [24]. The other source is the leakage from landfills and sewage treatment facilities, industrial waste systems and septic tanks [25].

Nonetheless, it is consensual that the most significant entry route for micropollutants into the aquatic environment is the release from wastewater treatment plants (WWTPs). Many natural and synthetic contaminants from domestic, hospital and industrial uses reach the WWTPs. Since these WWTPs are not specifically designed to remove these compounds, the treatment processes used by most WWTPs seem to be inefficient to remove organic compounds, the pollutants being released into the aquatic environment [26]. Afterwards, micropollutants resulting from many different pathways, described above, can be released into the surface water and groundwater, and once surface waters can be physically linked to groundwaters, one can contaminate another, even after soil or bank filtration [27]. Some of these chemicals are persistent in the environment, so it is possible that could be detected in tap waters, when surface or groundwaters are contaminated with such recalcitrant compounds and are used as raw water for DW supply. To overcome this possibility, the ultimate elimination step of micropollutants in raw water before DW distribution may take place during the treatment processes occurring at DW treatment plants (DWTPs).

Regarding to the knowledge about CECs in DWs, studies are still scarce and most countries do not have monitoring programs to routinely determine micropollutants in DW, owing to practical and economic difficulties. Thus, the majority of the occurrence data is published by academic research groups. The occurrence of micropollutants in DW will be further discussed in *Section 2* (*State of the art*) of the present thesis.

1.2.3. Public health risks

A great concern about the occurrence of micropollutants in the aquatic resources and the subsequent effects on humans and biota has been highlighted in the last few years. It is difficult to predict which environmental and public health implications may arise from the occurrence of CECs in freshwater ecosystems, since the concentrations usually found in the environment are lower than those able to cause direct negative effects [28]. For instance, concerning pharmaceuticals, toxicology studies have shown that they might have direct toxicity towards certain aquatic organisms [29]. The main problematic related to the frequent occurrence of recalcitrant compounds is the long term exposition that can lead to serious chronic effects, as reported by several studies [30, 31]. Their constant but imperceptible effects can gradually accumulate, finally leading to irreversible changes on both wildlife and human beings [32, 33].

There is a huge concern about the presence of certain classes of compounds in the environment. As example, the environmental contamination by antibiotics is alarming due to the probable development of resistance mechanisms by bacteria. This could subsequently compromise public health in terms of human treatment success, narrowing the therapeutic options that were available before. Another therapeutic class raising great alertness is steroid hormones, a group of highly active biological compounds able to induce the therapeutic effect at very low doses. Within this group, estrogens are the most usually found in the aquatic environment, existing either as natural or synthetic substances and acting as endocrine-disrupting compounds (EDCs) [34]. The effects of EDCs toward animals are well reported, for example, Kidd *et al*. [30] developed a 7-year experiment, verifying that the chronic exposure of fathead minnow to 5-6 ng L^{-1} of ethinylestradiol (EE2) led to feminization of males fish and altered oogenesis in females. EE2 is a synthetic estrogen present in oral contraceptive pills with proved estrogenic effects in fish. Some studies suggested that the effect of EDCs exposure on human health includes a decrease in male sperm count, an increase in testicular, prostate, ovarian and breast cancers and reproductive malfunctions [35]. The major concern is related to fetuses and newborn babies, because of their higher vulnerability [36]. Also the widely used antiepileptic carbamazepine showed to be lethal to zebrafish at 43 μg L^{-1}. Beyond that, it proved to be carcinogenic to rats, though not presenting mutagenic properties in mammals [37].

Regarding the pesticides, the impact of these contaminants in the environment and to the wild life is demonstrated by several injurious, such as the enhancement of the incidence of cancer, birth defects, genetic mutations, or other problems such as damages in the liver or in the central nervous system. Recently, studies conducted by Song *et al*. [38] suggested that relatively high doses of atrazine could exert reproductive toxicity to male rats. Sugeng

et al. [39] reported the "Hazard-ranking of agricultural pesticides for chronic health effects in Yuma County, Arizona", a study that classified some pesticides such as trifluralin, bensulide, maneb, endosulfan, and chlorpyrifos, as endocrine disruptors. Previous studies conducted by McKinney *et al*. [40], showed that the fungicides as penconazole and epoxyconazole could affect the thyroid, prostate, sex hormone balance and cause ovarian tumors.

1.3. European legislation

At the present time, one of the most important topics in the environmental field is the water quality. The increasing demand for water protection and treatment by environmental organizations and population in general, was one of the major reasons why the European Commission (EC) set water protection as one of its top work priorities.

Whilst some actions taken in the past by the European Union (EU), such as the Drinking Water Directive (DWD) and the Urban Waste Water Directive (UWWD), can properly be considered milestones, a renewed EU Water Policy was mandatory to address the increasing awareness of citizens and other implicated parties. Consequently, and as the outcome of a consultation process involving all interested parties, the Commission presented, in 2000, a proposal for a WFD, with the aim of identifying PSs with high risk to the aquatic ecosystems [9]. Hence, the WFD (Directive 2000/60/EC) was adopted as an operational instrument, setting the objectives for water protection. It presented a huge breakthrough in the EU water policy, aiming to achieve a good ecological and chemical status for all surface waters at the latest 15 years from the date of entry into force, i.e. 22 December 2000 [9]. In 2008, a list of 33 PSs/groups of PSs was established at Union level by the Directive 2008/105/EC, in the field of water policy. Environmental quality standards (EQS) were defined for these 33 PSs/groups of PSs and for other eight pollutants, based on available data of acute and chronic effects to aquatic environment and human health, being expressed as an annual average value (level providing protection against long-term exposure) and/or maximum allowable concentrations (level providing protection against short-term exposure) [41].

Even more recently, the Directive 2013/39/EU amending the Directives 2000/60/EC and 2008/105/EC, updated the WFD [7]. This Directive promotes the defensive action and the polluter pays principle, the identification of pollution causes, dealing with emissions of pollutants at the source, and the progress of innovative water/wastewater treatment technologies, avoiding expensive solutions. In particular, a significant improvement in the WFD was achieved by amending the list of PSs previously defined in 2008/105/EC, namely:

a) New PSs were identified;

b) EQS for newly identified substances were defined, which should be met by the end of 2027;

c) EQS for substances already identified were revised, which should be met by the end of 2021;

d) Biota EQS were defined for some existing and newly identified PSs.

The Directive 2013/39/EU includes 45 PSs/groups of PSs and also certain other pollutants with defined EQS to be considered. The list of PSs is exposed in the *Table 8, Appendix A1.*

During 2015, the Commission published the decision on the establishment of a watch list of substances for Union-wide monitoring in water bodies. The monitoring of those 10 substances (*Table 9, Appendix A2*) should generate high-quality data on their concentrations in the aquatic environment, improving the available data that is currently lacking.

1.4. Analytical methods for determination of micropollutants in drinking water

The presence of micropollutants in the aquatic environment, especially in DW, is considered an important issue in terms of human health safety [16]. In this context, it is important to set up fast, sensitive and reliable analytical methods that enable the determination of a wide range of these pollutants in DW, at residual levels usually found. The analytical challenge of measuring micropollutants at low concentrations in the environment has been a major research focus for scientists in the last decades [42]. With the development of analytical methodologies to detect trace chemicals in aqueous matrices, many studies have been focused on the development of methods for determination of these substances in different environmental matrices. Several techniques have been improved in order to achieve a high sensitivity and reproducibility for the detection of pollutants in the environment. Considering the wide resources and time consumption involved in this task, the novel developed analytical methods should meet the multiresidue or multiclass purposes, being able to determine trace levels of a large amount of compounds chemically heterogeneous and simultaneously reduce the cleanup and extraction steps [43, 44]. Many multiresidue analytical methods have been described in literature, mostly for wastewaters [14, 44]. Some of them refer to the detection of specific class of pollutants [45, 46], while the others intend to analyze a wider spectrum of compounds [6, 47]. The setup of a multiresidue method implies a thorough overview of the diverse steps involved in the analytical process. Beyond the separation and detection of

the target analytes, the usual sample preparation to cleanup the interferences and concentrate the target compounds, remains as one of the most important procedures of the analytical process during environmental analysis [48].

1.4.1. Extraction and concentration of water samples

Two main issues can be distinguished, during the development of a method for environmental matrices analysis: a) sample preparation; b) analytical separation and detection. Sample preparation is often highly time-consuming, comprising limited automated processes, which can jeopardize the reproducibility [49]. Thus, the employ of an accurate and precise sample preparation is crucial in the analysis of micropollutants in water.

The main goal of sample preparation is to obtain a sample extract enriched in target analytes and free of other components present in the matrix, as far as possible. Basically it encompasses the following main steps:

1) Extraction of low amounts of the target analytes from the sample matrix;

2) Concentration of those analytes;

3) Removal of other substances which may be co-extracted and simultaneously concentrated, consequently hampering the efficiency of the method [50, 51].

Entire effort in the development of this analytical procedure may result in considerable increase in the yield and quality of the results obtained [49]. The most important sample pre-treatment techniques will be discussed in the following section.

- **Solid phase extraction (SPE)**

Solid phase extraction (SPE) is the most popular sample preparation technique for environmental samples. The principle of this technique involves partitioning between a liquid phase containing the analytes and a solid sorbent phase.

The SPE procedure consists in five different steps: conditioning, sample loading, washing, dryness and elution. The objectives of these stages are the extraction, concentration of the target analytes, elimination of interferences, removal of the residual water and finally desorption of the analytes. Owing to its high versatility, SPE is used for several purposes, such as purification, trace enrichment, desalting, derivatization of analytes in the cartridge and fractionation of the sample extract in different groups of compounds. However, SPE has some drawbacks, such as the loss of more polar compounds during sample percolation; the co-extraction of matrix interferences; the clogging of the filters; the long period for the extraction of large volumes; the significant consumption of organic

solvents; the incomplete desorption of target analytes; the loss of volatile compounds and; the incomplete recovery of the dry extract [43, 52].

To achieve optimal SPE extraction conditions, the selection of the sorbent is an important factor because it will affect the selectivity, affinity and capacity [52]. The choice depends strongly on the nature of the analytes and their physical and chemical properties, which define the interactions with the selected sorbent. Nevertheless, results also depend on the sample matrix and its interactions with both sorbent and analytes [52]. The sorbent can interact with analytes by different type of interactions: hydrophobic (non polar-non polar, van der Waals), hydrophilic (polar-polar, hydrogen bonding, dipole-dipole, dipole-induced dipole), cationic, anionic and selective antigen-antibody interactions. Some examples of these sorbents are displayed in *Figure 3*.

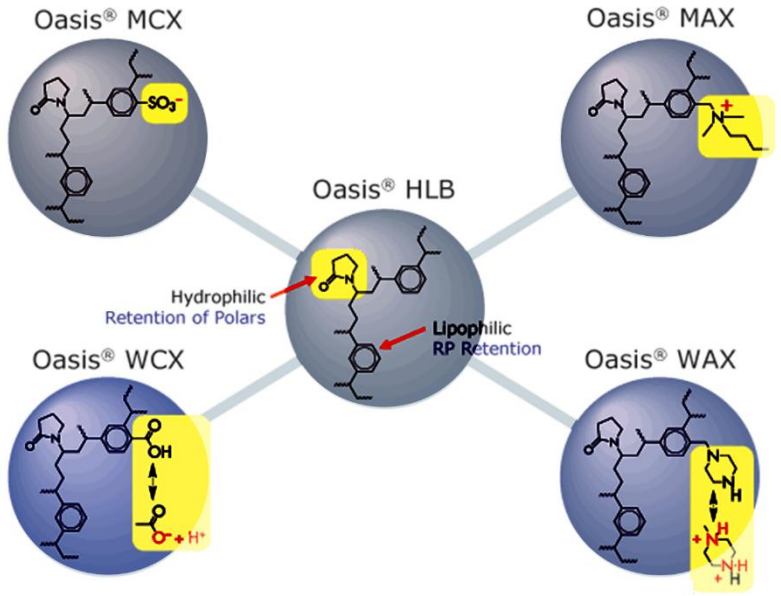


Figure 3 - Commercial brands of Oasis ® SPE extraction cartridges constituted by polymeric adsorbents, frequently utilized in the extraction of micropollutants from water samples (Adapted from ref. [53]).

It is still important to refer that the extraction efficiency is affected, not only, by the type of sorbent, but also by the solvent used, sample pH and sample volume loaded. These parameters have to be carefully optimized in order to obtain successful results [14].

- **Other extraction techniques**

Besides SPE, which is the most used technique, solid phase microextraction (SPME) has also earned huge acceptance in this field, presenting further development potential. In contrast, more conventional techniques are less employed nowadays. This is the case of liquid-liquid extraction (LLE), which presents several negative aspects, including the

emulsion formation, the use of large volumes of toxic and environmentally harmful solvents, the time consumption and high cost. Liquid phase microextraction is a solvent miniaturized sample pre-treatment mode of LLE, requiring some µL of organic solvent to concentrate analytes [54], however having a low precision [55]. Recent extraction techniques include the dispersive liquid-liquid microextraction and stir membrane liquid-liquid microextraction, both presenting high extraction efficiencies [52].

1.4.2. Separation and detection of micropollutants

Highly sensitive methods are required to determine the trace levels of micropollutants in environmental matrices. The separation of organic compounds is normally performed either by liquid or gas chromatography (LC or GC, respectively), according to the volatility, polarity and thermal stability of the analytes. Whilst volatile or semi-volatile compounds may be analyzed by GC, more polar or thermolable non-polar compounds are analyzed by LC, with no need of prior derivatization [51]. Nowadays, fast and high resolution LC systems are available, with high resolution and separation efficiency, as ultra-high performance liquid chromatography (UHPLC), that enable to work at pressures up to 1300 bar using sub-2-µm particle packed columns [56].

The detection of a wide range of organic contaminants at trace or ultra-trace concentrations, including micropollutants in DW, is a challenge. Hyphenated chromatography-mass spectrometry techniques are presently the methods of election for this type of analysis due to the notable improvement in method detection limits achieved by these methodologies [56]. Mass spectrometry (MS) is an instrumental technique based on the separation of ions according to their mass ratios (m/z), in the gas phase and in vacuum conditions. Basically, a mass spectrometer is constituted by an ion source, a mass analyzer and a detector (*Figure 4*) [57]. After the ionization process occurring in the ion source, the molecular ions formed can suffer an additional fragmentation and are then separated in the mass spectrometer, according to their m/z to be finally detected.

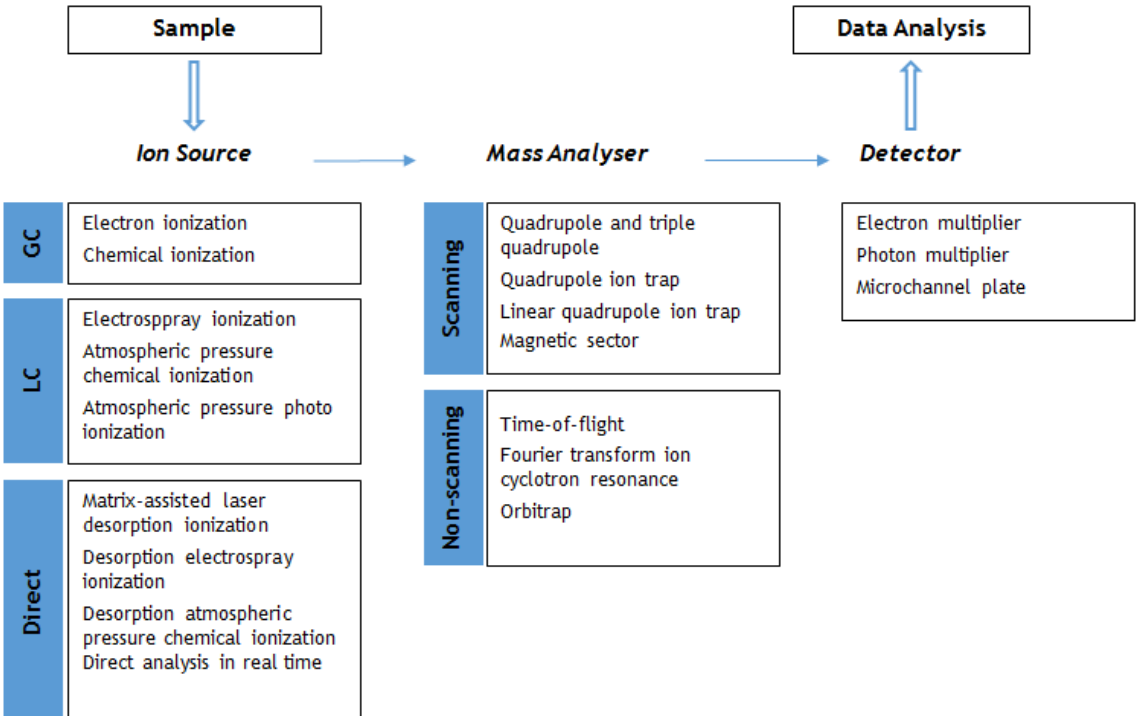


Figure 4 - Overview of mass spectrometric techniques (Adapted from ref. [57]).

The quadrupole represents the most popular mass analyzer due to its relatively low cost, ruggedness, reliability and simplicity of operation. Different mass analyzers can be coupled and form the so-called hybrid instruments. Triple quadrupole (QqQ) is the most usual hybrid instrument (*Figure 5)* and consists of two single quadrupoles (Q1 and Q3) with a collision cell (Q2) in between. The molecular ions are sent from the ion source to the first quadrupole (Q1), where the precursor ion is selected for fragmentation in the collision cell (Q2, usually a hexapole). The product ions are then separated in the second quadrupole (Q3) and recorded by the detector [57]. Afterwards, the detector allows to monitor the ion current and finally records the data as mass spectra.

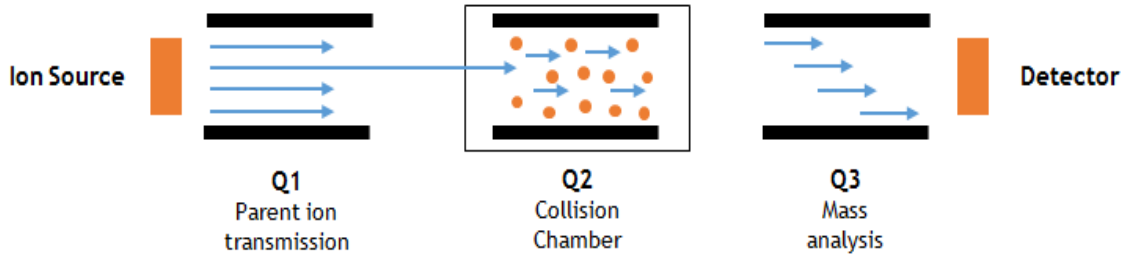


Figure 5 - Triple quadrupole (QqQ) mass analyzer: In MS/MS mode, the precursor ions are selectively transmitted to the collision chamber and fragmented, and the resulting product ions are resolved in the third quadrupole (Adapted from ref. [58]).

1.5. Treatment of micropollutants in drinking water

As previously mentioned, the conventional water treatments are not enough to remove the micropollutants present in water, independently from the origin. Therefore, the development of alternative technologies that enable the efficient removal of these pollutants is imperative.

Advanced oxidation processes (AOPs) are defined as oxidation methods occurring in the aqueous phase and based upon the generation of highly reactive species such as (primarily but not exclusively) hydroxyl radicals ($^{\bullet}OH$). These radicals present a high oxidation potential (E^0 = 2.80 V, relatively to the normal hydrogen electrode) and are responsible for the oxidation and mineralization of almost any organic molecule, yielding CO_2, H_2O and inorganic ions as final products, due to their strong and unselective oxidative power [59]. Thus, AOPs can be used to chemically decompose pollutants into harmless end-products, otherwise not removed by conventional treatment processes [60]. Over the past 30 years, the research and development of new AOPs has been huge, particularly due to the diversity of technologies involved and due to the areas of potential application [61].

The most known AOPs are ozonation, heterogeneous photocatalysis, the Fenton process, wet peroxide oxidation and wet oxidation. Some of them can be assisted by active catalysts and/or by hydrogen peroxide [62]. It is also possible to combine different AOPs with the aim of increasing the overall treatment efficiency. Photolysis (UV) and ozonation (O_3) are the most used processes in the particular case of DW treatment [63, 64].

Regarding DW treatment, UV radiation is widely used to induce photoreaction of micropollutants, such as pharmaceutical compounds. Similarly, ozone is also seen as an efficient oxidant for the purification of DWs [65]. Both treatments have a complementary antimicrobial effect, useful for DW disinfection. However, they cannot be conceptually considered as AOPs regarding the exclusive formation of $^{\bullet}OH$ over all conditions applied (e.g. ozonation at low pH proceeds through the pollutant molecule direct attack by ozone).

Other treatments such as O_3/H_2O_2, O_3/UV and Fenton's reagent may be applied to DW. However, these processes are more often applied to wastewaters [61, 66, 67]. In the present work, single photolysis or ozonation were applied at lab-scale to DW from different sources; thus major relevance will be given to these two processes in the following sections.

1.5.1. Photolysis

UV photolysis has been one of the most widely studied chemical transformation processes for the removal of micropollutants in water [68]. UV photolysis is a process that degrades organic compounds through the incidence of radiation (200-400 nm). It involves the interaction of artificial or natural light (UV portion) with the target molecules, which can absorb a quantum of light energy or photon in their ground state and transit to a higher-energy state, following different reaction pathways. These reactions lead to the degradation of compounds and might ultimately result in their mineralization, forming CO_2, H_2O and inorganic ions. The efficiency of this process depends on some factors, such as the absorbance spectrum of the micropollutant, the quantum yield of photolysis and the water matrix [61, 68].

An extensive range of UV lamps may be applied to degrade organic compounds. Low pressure (LP) and medium pressure (MP) UV mercury lamps are conventional UV sources applied for water disinfection due to their germicidal effect. LP lamps emit predominantly radiation at 254 nm while MP lamps emit radiation at 200−500 nm. Although the use of both LP and MP lamps is already implemented in some DWTPs for disinfection, LP lamps have the advantages of lower by-products formation, energy demand, as well as operating costs [68]. UV treatment has usually been employed for the disinfection of DW with the advantage, compared to chlorination, of minimizing the formation of any regulated disinfection by-products [61].

1.5.2. Ozonation

Ozone is a powerful oxidizing agent and due to its high oxidation and disinfection potential, has recently received much attention in water treatment technology. The chemistry of ozone in aqueous solution is complex. Molecular ozone can oxidize water impurities via direct, selective reactions or can undergo decomposition by a chain reaction mechanism, resulting in the production of free $^{\bullet}OH$ [59]. The chemical properties of ozone depend on the structure of the molecule, which is represented in *Figure 6*.

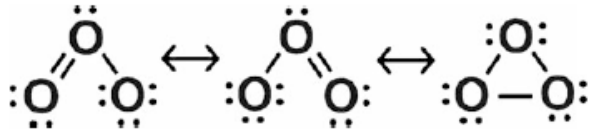


Figure 6 – Resonance structures of the molecule of ozone [59].

Due to its structure, molecular ozone can react as a dipole, an electrophilic or nucleophilic agent. As a result of its high reactivity, ozone is very unstable in water. The half-life time

of molecular ozone depends on several operating conditions, such as pH, water temperature and concentration of organic and inorganic compounds in water [62]. Ozone decomposition proceeds through the following five-step chain reactions (Eqs. 1-5) [59]:

$$O_3 + H_2O \rightarrow 2HO^{\bullet} + O_2 \quad (1)$$

$$O_3 + OH^- \rightarrow O_2^{\bullet -} + HO_2^{\bullet} \quad (2)$$

$$O_3 + HO^{\bullet} \rightarrow O_2 + HO_2^{\bullet} \leftrightarrow O_2^{\bullet -} + H^+ \quad (3)$$

$$O_3 + HO_2^{\bullet} \underset{\rightarrow}{\leftarrow} 2O_2 + HO^{\bullet} \quad (4)$$

$$2HO_2^{\bullet} \underset{\rightarrow}{\leftarrow} O_2 + H_2O_2 \quad (5)$$

Ozone is used in disinfection, taste, odor and color control, oxidation of inorganic pollutants (iron, manganese), oxidation of organic micro and macropollutants, as well as for the improvement of coagulation [69]. The addition of the oxidant in the DW treatment have two purposes: pre-oxidation and/or intermediate oxidation. Generally, pre-oxidation is applied for the elimination of inorganic compounds, color, taste, odor, turbidity and suspended solids. During this step, the partial degradation of natural organic matter and inactivation of microorganisms occurs, as well as the coagulation–flocculation–decantation step enhancement takes place. Intermediate oxidation has the aim of the degradation of micropollutants, the removal of trihalomethanes precursors and increase of biodegradability [70].

1.6. Objectives

The aim of this work was the development of an analytical method based on SPE followed by UHPLC-MS/MS, for the fast and simultaneous determination in DW, of 23 micropollutants (14 pharmaceuticals, 1 metabolite, 7 pesticides and 1 industrial compound), some of them defined in the Directive 2013/39/EU and in the watch list of Commission Decision 2015/495/EU. The study of two chemical processes photolysis and ozonation to remove these pollutants were also focused on this thesis. Thus, the specific objectives of this work can be listed as:

- Development and optimization of a SPE-UHPLC-MS/MS method;
- Validation of the SPE-UHPLC-MS/MS method;
- Analysis of DW samples from different sources (tap water, fountain water and well water);
- Study of photolysis and ozonation to remove micropollutants from DW.

2. State of the art

Although the presence of micropollutants in DW is nowadays a colossal human health problem, a limited number of analytical methods for the simultaneous determination of a wide range of micropollutants in DW can be found in the literature, *Table 1*.

Table 1 - Analytical methods developed for analysis of micropollutants in DW.

Sample Preparation	Chromatographic Conditions	Target Analytes	Observations	Ref.
SPE Cartridges: Oasis® HLB; Conditioning: 4 mL of methanol + 6 mL of ultrapure water; Sample loading: 200 mL; Elution: 5 mL of methanol.	*LC/MS-MS* using triple quadrupole Column: Agilent Zorbax Eclipse Plus C_{18} (100 mm×2.1 mm i.d.); Mobile phase: water (0.1% formic acid)/acetonitrile (90/10, v/v); Flow rate: 0.2— 0.3 mL min^{-1}.	70 pharmaceutical compounds	Only carbamazepine was found at 5 ng L^{-1}.	[42]
SPE of pharmaceuticals Cartridges: Oasis® HLB; Conditioning: 3 mL of methanol + 3 mL of ultrapure water; Sample loading: 240 mL; Elution: 5 x 3 mL of methanol. SPE of pesticides Cartridges: C_{18}; Conditioning: 3 mL of methanol + 3 mL of ultrapure water (acidified with 0.1% formic acid); Sample loading: 140 mL; Elution: 3 x 3 mL ethylacetate + 1 x 3 mL methanol	*LC/MS-MS* using triple quadrupole Column: XTerras MS C_{18} (100 mm ×3 mm i.d., 3.5 µm); Mobile phase: a gradient of water (0.1% formic acid) and MeOH:H_2O (90:10 v/v); Flow rate: 3 mL min^{-1}.	8 pharmaceutical compounds and 9 pesticides	Atenolol, paracetamol, diazepam, ibuprofen, carbofuran and diuron were detected, but not quantified. Atrazine was quantified (0.0093–0.081 µg/L^{-1}).	[48]
SPE Cartridges: C_{18}; Conditioning: 5 mL of methanol + water at pH 2 (acidified with formic acid) + water; Sample loading: 1000 mL; Elution: 6 mL of acetonitrile (acidified with 0.005% formic acid) + water at pH 3.8 (formic acid) + methanol (5% ammonium hydroxide).	*UHPLC-LTQ-orbitrap* Column: Acquity BEH C_{18} (50 mm×2.1 mm i.d.,1.7 µm); Mobile phase: a gradient of water (0.005% formic acid) and acetonitrile (0.005% formic acid); Flow rate: 0.5 mL min^{-1}.	8 pharmaceutical compounds and a metabolite of caffeine	Metformin, cimetidine, caffeine, paraxanthine, erythromycin, fluoxetine and gemfibrozil were detected (0.2–0.3 µg L^{-1}).	[71]

Table 1 – (Continued)

Sample Preparation	Chromatographic Conditions	Target Analytes	Observations	Ref.
SPE Cartridges: Oasis® MCX; Conditioning: 5 mL acetone + 5 mL water; Sample loading: 100 mL; sample adjusted to pH 3 (HCl); Washing: 6 mL water (pH 3); Elution: 8 mL methanol (5% ammonium hydroxide).	*LC-MS/MS* using triple quadrupole Column: RP-18 Waters XTerra (100×2.1 mm i.d., 3.5 μm); Mobile phase: a gradient of 2 mmol L^{-1} ammonium acetate in water and 2 mmol L^{-1} ammonium acetate in methanol; Flow rate: 0.2 mL min^{-1}.	13 pharmaceutical compounds	Acetylsalicylic acid, carbamazepine, clofibric acid and sulfamethoxazole were detected.	[72]
SPE Cartridges: Oasis® HLB; Conditioning: 6 mL methanol + 2 mL of Milli-Q water + 2 mL Milli-Q water at pH 2 + 6 mL Milli-Q water; Sample loading: 250 mL; Washing: 5 mL Milli-Q; Elution: 5 mL methanol + 3 mL acetone-methanol.	*LC/MS-MS* using triple quadrupole Column: Supelco C_{18} (150 × 2.1 mm i.d., 5 μm) Mobile phase: a gradient of water (0.1% formic acid) and acetonitrile (0.1% formic acid); Flow rate: 0.25 mL min^{-1}.	16 pharmaceutical compounds	7 pharmaceutical compounds were found (caffeine, carbamazepine, ibuprofen, sulfamethoxazole, lincomycin, acetaminophen, triclosan).	[16]
SPE Cartridges: Oasis® HLB and MCX; Conditioning: 5 mL methanol + 5 mL HPLC grade water for Oasis HLB and + 5 mL of acidified HPLC grade water (pH 2.5–3) for Oasis MCX, respectively; Sample loading: 50 mL; Elution: 6 mL of pure methanol for Oasis HLB; 3 mL of methanol + 3 mL of 5% of NH_4OH in methanol for Oasis MCX.	*UHPLC-QqLIT MS/MS* Column: Acquity HSS T3 (50 mm× 2.1 mm i.d., 1.8 μm) Mobile phase: a gradient of water (10 mM formic acid/ammonium formate, pH 3.2) and methanol; Flow rate: 0.5 mL min^{-1}.	81 pharmaceutical compounds and some of their main metabolites	15 pharmaceuticals were detected up to 21 ng L^{-1} (ibuprofen, indomethacine, salicylic acid, propyphenazone, gemfibrozil, atorvastatin, carbamazepine, diazepam, ranitidine, nadolol, hydrochlorothiazide , torasemide, losartan, valsartan, azithromycin and sulfamethoxazole).	[6]
SPE Cartridges: Oasis® HLB; Conditioning: methanol and water; Sample loading: 500 mL after addition of 2 mL of a 5 mg L^{-1} EDTA solution; Elution: 2 x 4 mL of methanol.	*UPLC using triple Quadrupole* Column: Acquity BEH C18 (2.1×50 mm i.d., 1.7 μm) Mobile phase: a gradient of water (0.01 mM ammonium acetate or 0.5% formic acid) and methanol; Flow rate: 0.03 mL min^{-1}.	31 pharmaceutical compounds	7 pharmaceuticals were detected up to 50 ng L^{-1} (caffeine, carbamazepine, atenolol, sulfadiazine, sulfapyridine, sulfamethoxazole and erythromycin).	[73]

Concerning sample preparation, it is possible to conclude that the cartridges Oasis® HLB and the solvent methanol are the most used. Additionally, LC-MS/MS is the technique per excellence for micropollutants determination in DW samples. Furthermore, the pharmaceutical compounds are the most studied class of micropollutants, being carbamazepine, caffeine, ibuprofen and sulfamethoxazole the most detected compounds.
Since the progressive increase of micropollutants in water is a major problem, their removal from different water matrices has been studied by several authors; however, available data for DW is limited (*Table 2*). Some authors studied the removal of micropollutants by chemical processes at lab-scale, but also at pilot-scale and real scale, i.e. in conventional DWTPs.

Table 2 - Removal of micropollutants in DW by chemical processes.

Process	Target Analytes	Operating Conditions	Observations	Ref.
Ozonation	Clofibric acid, ibuprofen and diclofenac	Lab-scale **1 -** C_i = 2 μg L^{-1}; $[O_3]$ = 3.7 mg L^{-1}; t = 10 min; **2 -** C_i = 2 μg L^{-1}; $[O_3]$ = 5.0 mg L^{-1}; t = 10 min.	**1** - 90% of clofibric acid, 90% of ibuprofen and 100% of diclofenac were removed; **2 -** 97.9% of clofibric acid, 99.4% of ibuprofen and 100% of diclofenac were removed.	[74]
Ozonation	Carbamazepine, bezafibrate, diclofenac and clofibric acid	Lab-scale C_i = 1 μg L^{-1}; $[O_3]$ = 0.5 − 3.0 mg L^{-1}; t = 20 min.	97% of carbamazepine and diclofenac were eliminated using ozone at 0.5 mg L^{-1}. Bezafibrate was removed by 50% using ozone at 1.0–1.5 mg L^{-1} while 90% was removed using 3.0 mg L^{-1}. Only 10–15% removal of clofibric acid was achieved with 0.5 mg L^{-1} of ozone. At higher ozone concentration (2.5–3.0 mg L^{-1}), 40% of clofibric acid was removed.	[75]

Table 2 – (Continued)

Process	Target Analytes	Operating Conditions	Observations	Ref.
Ozonation	Carbamazepine, caffeine, cotinine and atrazine	Pilot-scale pH = 7.5; $[O_3]$ = 1.5 - 2.0 mg L^{-1}.	Low concentrations (ng L^{-1} range) were detected and depending on the season, the water condition and the treatment, residual levels of carbamazepine, cotinine and caffeine, as well as atrazine, still remained in the finished drinking water, even after ozonation.	[76]
Ozonation	55 pharmaceuticals (e.g. carbamazepine, fluoxetine, metoprolol, clopidrogel, venlafaxine and warfarin), hormones and metabolites (e.g. norfluoxetine)	DWTP C_i = 0.2 mg L^{-1}; 4 ozone chambers; $[O_3]$ = 5 mg L^{-1}; t = 15—20 min.	Metoprolol and venlafaxine persisted through treatment. The other compounds were completely removed (> 99%).	[77]
Ozonation UV	4 beta blockers (e.g. metoprolol), 1 antiepileptic drug (e.g. carbamazepine), 1 lipid regulator (bezafibrate), 4 anti-inflammatories (e.g. diclofenac) and 3 fluoroquinolones	Pilot-scale Ozonation: C_i = 0.3 mg L^{-1}; $[O_3]$ = 1.0—1.3 mg L^{-1}; t = 10 min. UV: UV dose = 250 J m^{-2}.	Most pharmaceuticals (e.g. carbamazepine and diclofenac) were eliminated by ozonation. Metoprolol and bezafibrate still remained in the finished drinking water (60% and 70%, respectively). After UV irradiation metoprolol and bezafibrate were not detected.	[63]

By the analysis of the different works, it was possible to verify that the compounds most studied at lab and pilot scale were clofibric acid, diclofenac and carbamazepine, while in DWTP a larger group of micropollutants was considered.

Ozonation was the most applied process in DW treatment. According to the scale of the treatment, the operational conditions are different. The ozone dose ranges 0.5 to 15 mg L^{-1} and the time of experiment 10 to 20 min. Regarding the results, ozonation leads to high degradation rates for the different micropollutants studied, in most of the cases. However, the ozone dose applied was not sufficient for total removal of some pollutants.

UV applied after ozonation showed to be effective for the removal of micropollutants, which were not removed by ozone.

3. Experimental

3.1. Chemicals and materials

In this work, 23 micropollutants of different groups (7 pesticides, 1 industrial compound, 14 pharmaceuticals and 1 metabolite) were studied. *Table 3* shows the class, structure, molecular weight (Mw) and p*K*a of the target micropollutants. All reference standards (diclofenac sodium, tramadol hydrochloride, azithromycin dihydrate, clarithromycin, trimethoprim, warfarin, clopidrogel hydrogen sulfate, metoprolol tartrate, atorvastatin calcium salt trihydrate, bezafibrate, carbamazepine, citalopram hydrobromide, venlafaxine hydrochloride, fluoxetine hydrochloride, norfluoxetine oxalate, alachlor, atrazine, simazine, isoproturon, chlorfenvinphos, pentachlorophenol, clofibric acid and perfluorooctanesulfonic acid; > 98% purity) were purchased from Sigma-Aldrich (Steinhein, Germany).

Table 3 - Compounds studied in the present work: class, structure, molecular weight (Mw) and p*K*a.

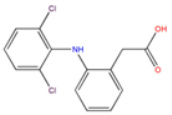
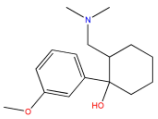
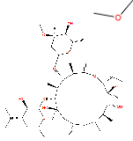
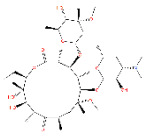
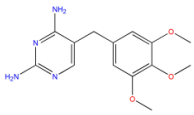
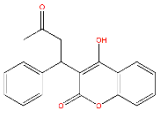
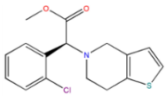
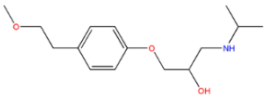
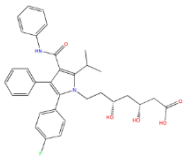
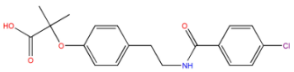
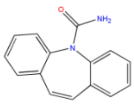
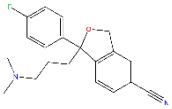
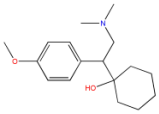
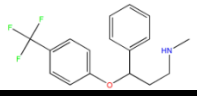
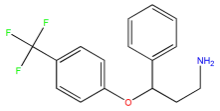
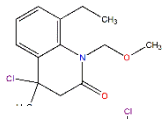
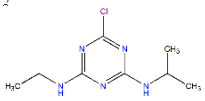
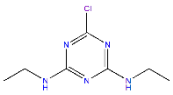
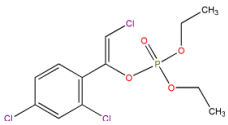
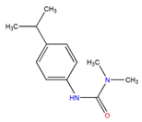
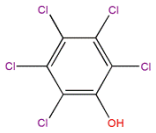
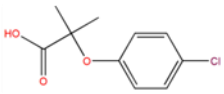
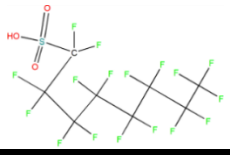
Class and sub class	Analyte	Structure	Mw (g mol^{-1})	p*K*a
Pharmaceuticals				
Anti-inflammatories	Diclofenac		296.14	4.15
	Tramadol		263.38	9.41
Antibiotics	Azithromycin		748.51	8.74
	Clarithromycin		747.95	8.99
	Trimethoprim		290.32	7.12
Anticoagulant	Warfarin		308.33	4.50
Antiplatelet agent	Clopidogrel		321.06	5.14
Beta-blockers	Metoprolol		267.36	9.67
Lipid regulators and cholesterol lowering statin drugs	Atorvastatin		558.25	4.46
	Bezafibrate		361.82	3.61
Psychiatric drugs	Carbamazepine		236.27	13.94
	Citalopram		324.16	9.78
	Venlafaxine		277.4	9.40
	Fluoxetine		309.33	9.80

Table 3 - Continued.

Class and sub class	Analyte	Structure	Mw (g mol^{-1})	p*Ka*
Metabolite	Norfluoxetine		295.3	9.77
Pesticides				
Chloroacetanilide	Alachlor		269.77	0.62
Triazine	Atrazine		215.68	4.14
	Simazine		201.66	1.62
Organophosphorus	Chlorfenvinphos		359.57	n.a.
Phenylurea	Isoproturon		206.28	n.a.
Organochlorine	Pentachlorophenol		266.34	4.73
Herbicide	Clofibric acid		214.65	3.00
Industrial compound	Perfluorooctanesulfonic acid		500.13	0.14

Stock solutions of approximately 1000 mg L^{-1} of each pollutant were prepared in methanol, ethanol or acetonitrile, depending on the solubility of each standard. Two working standard solutions of 200 µg L^{-1} and 20 µg L^{-1} were prepared by diluting each stock solution in ethanol. Surrogate standards (ketoprofen-d3, fluoxetine-d5 solution and atrazine-d5) were purchased from Sigma-Aldrich (Steinhein, Germany). Individual isotopically labeled internal standards of ketoprofen-d3 and atrazine-d5 were prepared at a concentration of 1000 mg L^{-1}, by dissolving 10 mg of each standard in methanol. A working solution containing 10 mg L^{-1} of each isotopically labeled internal standard was prepared by dilution in ethanol.

Ethanol (HPLC grade) was purchased from Fisher Scientific UK Limited (Leicestershire, UK). Methanol and acetonitrile (MS grade) were acquired from VWR International (Fontenay-sous-Bois, France). Ammonium acetate, ammonium hydroxide 25%, sulphuric acid and formic acid were purchased from Merck (Darmstadt, Germany). Ultrapure water was supplied by a Milli-Q water system. HPLC grade solvents were filtered with 0.22 µm nylon membrane filters (Membrane Solutions, Texas, USA). The SPE cartridges tested were Oasis® HLB (Hydrophilic-Lipophilic-Balanced), Oasis® MCX (Mixed-mode Cation eXchange) and Oasis® MAX (Mixed-mode Anion-eXchange) (150 mg, 6 mL), purchased from Waters (Milford, Massachusetts, USA). A pH meter pHenomenal® pH 1100L (VWR, Germany) was used for the pH adjustments.

3.2. Solid phase extraction

Tap waters were collected from the water supply network of Porto (Portugal) to be used as matrix for the SPE optimization and validation of the method. The vacuum extraction and drying devices LiChrolut® used for SPE procedure (*Figure* 7) were acquired from VWR (Merck Millipore, Billerica, MA, USA).

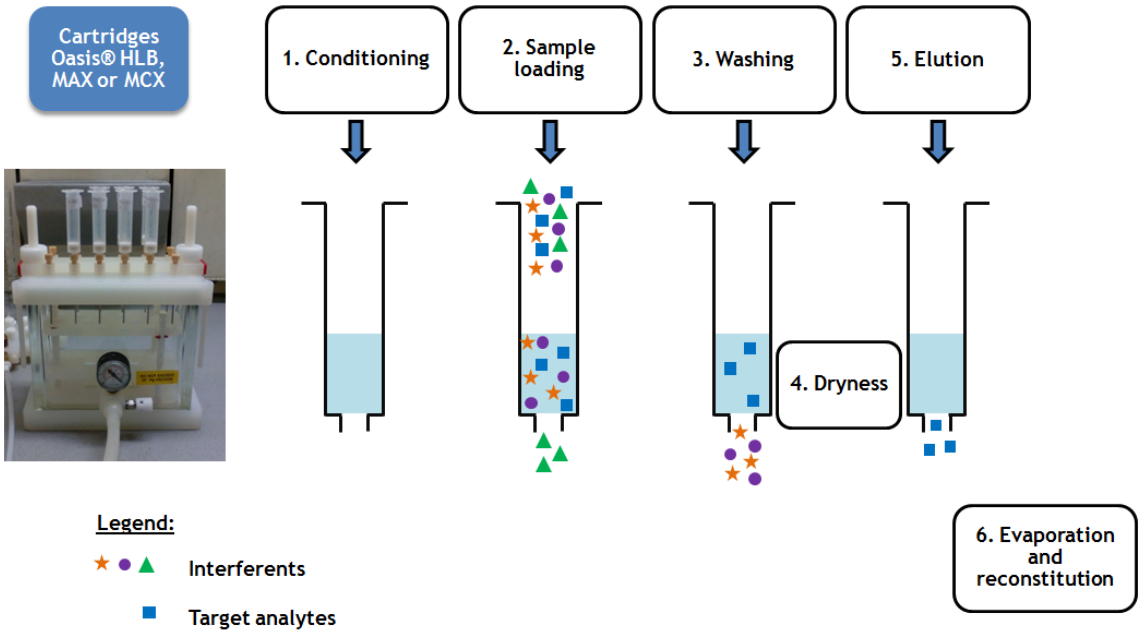


Figure 7 - Schematic representation of SPE procedure.

Oasis® HLB, MCX and MAX cartridges (150 mg, 6 mL) were tested to assess the best performance for the overall compounds. Oasis® MAX and MCX cartridges were conditioned sequentially at a flow rate of 1 mL min^{-1}, with 4 mL of methanol and 4 mL of ultrapure water. For HLB cartridges, the conditioning was performed at the same flow with 4 mL of

methanol or ethanol and 4 mL of ultrapure water. The sample pH was optimized for HLB cartridges, by comparing the recoveries achieved with initial sample pH 3, 7 and 9. For MAX and MCX SPE procedures, samples were alkalinized to pH 9 or acidified to pH 3, before loading. The pH adjustments were done with ammonium hydroxide and sulphuric acid. Sample loading was carried out with 250 mL of tap water blanks and spiked (35 ng L^{-1}) tap water samples at a constant flow rate of 10 mL min^{-1}, using the vacuum manifold unit connected to a vacuum pump. The washing step was performed with 4 mL of ultrapure water, 5% ammonium hydroxide aqueous solution, or 2% formic acid aqueous solution, for HLB, MAX and MCX, respectively. After washing, the cartridges were dried during 45 min under vacuum. The elution step was performed at a flow rate of 1 mL min^{-1} with: 4 mL methanol or ethanol for Oasis® HLB cartridges, 4 mL of methanol to extract the neutral compounds and weak bases in the case of Oasis® MAX and neutrals and weak acids in the case of Oasis® MCX. A second elution was performed for mixed-mode cartridges Oasis® MAX and MCX, respectively with a 2% formic acid methanolic solution (elution of acids) or 5% ammonium hydroxide methanolic solution (elution of basics). The LiChrolut® drying device was coupled to the vacuum extraction unit to evaporate the extracts to dryness with a gentle nitrogen stream. The dry residues were reconstituted in 300 µL of ethanol and the ethanolic extracts were filtered using 0.22 µm polytetrafluoroethylene (PTFE) syringe filters (Membrane Solutions, Texas, USA). To evaluate the breakthrough volume, sample loading was tested with four volumes of tap waters, namely 100, 250, 500 and 1000 mL, using the optimized SPE procedure and both non-spiked samples and 35 ng L^{-1} spiked. In order to improve the recovery rates, the quelating agent ethylenediaminetetraacetic acid (EDTA) (0.2 M) was tested as well as two dechlorination agents, ascorbic acid (10 mg L^{-1}) and sodium thiosulfate (6 g L^{-1}).

3.3. Ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) analysis

A Shimadzu Corporation apparatus (Tokyo, Japan), *Figure 8*, was employed to perform the chromatographic analysis. It consists on a UHPLC equipment (Nexera) with two pumps (LC-30AD), an autosampler (SIL-30AC), an oven (CTO-20AC), a degasser (DGU-20A 5R) and a system controller (CBM-20A), coupled to a triple quadrupole mass spectrometer detector (Ultra Fast Mass Spectrometry series LCMS-8040), with a LC Solution Version 5.41SP1 software. A Kinetex™ 1.7 µm XB-C18 100 Å column (100 × 2.1 mm, i.d.) supplied by Phenomenex, Inc. (California, USA) was used.

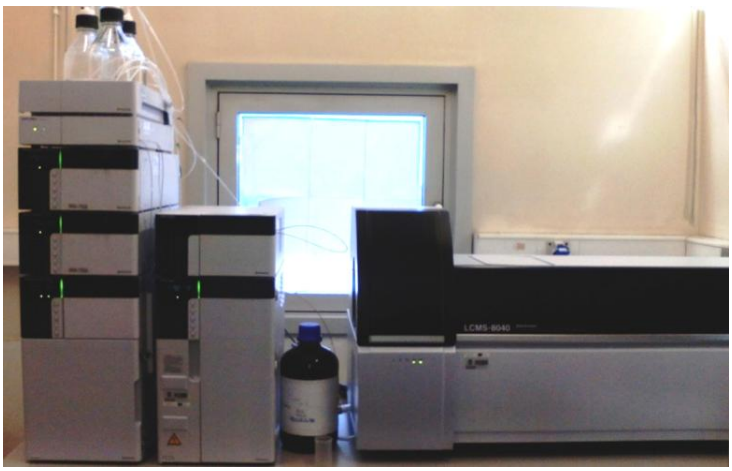


Figure 8 - Equipment used to LC-MS/MS analysis.

The optimized mobile phase was ethanol/water (70/30, v/v) performed at isocratic mode at a flow rate of 0.20 mL min^{-1}. Column oven and autosampler temperatures were set at 35 ° C and 4 ° C, respectively. Volume of injection was 5 µL. An electrospray ionization source was used operating in both positive and negative ionization modes.

Each individual standard stock solution at 1000 µg L^{-1} was injected without column to choose the precursor ion through full scan mode, to select the two most abundant fragments and to optimize the following mass spectrometer parameters: declustering potential, collision energy and collision cell exit potential. Quantification was performed by selected reaction monitoring (SRM), selecting the two SRM transitions between the precursor ion and the referred two most abundant fragment ions of each compound, with a scan time of 100 ms per transition, being the most abundant used for quantification purposes and the second most abundant for identity confirmation (*Table 4*). Capillary voltage (0.5, 1.5, 2.5, 3.5 and 4.5 kV), drying gas flow (10.0, 12.5 and 15.0 L min^{-1}), nebulizing gas flow (1.0, 1.5, 2.0, 2.5 and 3.0 L min^{-1}), desolvation temperature (200, 225, 250, 275 and 300 ° C) and source temperature (200, 250, 300, 350, 400 and 450 ° C) were optimized, through the injection of a working standard solution with the target compounds at 50 µg L^{-1}. The collision induced dissociation gas (CID) was argon at 230 kPa.

3.5. Method validation parameters

The SPE-UHPLC-MS/MS method validation was performed according to the international guidelines [78] and previous works [79-81], through the evaluation of the following parameters: selectivity, linearity and range, limits of detection and quantification, accuracy, recovery and precision. Chromatograms of non-spiked tap waters (blank extracts), standards extracted from the spiked tap waters at 35 ng L^{-1} and an ethanolic solution containing all the standards (at a concentration corresponding to the theoretical

concentration after SPE, i.e. 250/0.3 fold higher) were compared. For recovery experiments, three quality control (QC) standard solutions were prepared, in triplicate in three consecutive days, by extracting tap water samples spiked with three different concentrations (3.5, 20 and 35 ng L^{-1}). The peak areas of the standards extracted from the spiked tap waters were compared with those of ethanolic solutions containing all the standards at the theoretical concentrations of totally recovered extracts, to assess the recovery of each SPE procedure. For target compounds detected in the blank matrix, the peak areas were subtracted from those obtained with the spiked matrix.

The internal standard calibration method was used to define the linearity and range for each target analyte. Triplicates of 250 mL tap water samples spiked with seven different standard concentrations (0.75, 1.5, 2.0, 4.0, 8.0, 20 and 40 ng L^{-1}) were prepared and 10 µL of a working internal standards solution of 10 mg L^{-1} was added to each sample. These standard solutions were extracted by the optimized SPE procedure and reconstituted in 300 µL of ethanol to perform the calibration curves, by injecting 5 µL in the UHPLC apparatus. Instrument detection (IDL) and quantification (IQL) limits were calculated from spiked samples through the signal-to-noise (S/N) ratio of 3.3 and 10 for IDL and IQL, respectively. Method detection (MDL) and quantification (MQL) limits were determined through the division of the respective instrument limit by the pre-concentration factor. The three triplicate QC solutions, described above, were also used to evaluate the accuracy of the method as well as the precision (intra- and inter-batch). The concentrations of the analytes in the SPE extracts calculated using the calibration curves was compared with the nominal concentration, in percentage, to determine the accuracy. Precision of the method was expressed through the relative standard deviation (RSD) of the intra-batch and inter-batch replicate analyses [82-84]. In order to evaluate the possible carry-out effect, ethanol was injected after each set of triplicates.

3.6. Matrix effect evaluation

The post-extraction addition method was used to assess the matrix effect [81, 84]. The method was carried out on tap water samples, by analyses of three post-spiked (29.17 µg L^{-1}) extracts of blank water samples and comparison with three extracts of non-spiked samples, using the optimized SPE procedure. The matrix effect (ME) was calculated as the ratio of the peak areas obtained for blanks extracts spiked after extraction, subtracting those of the non-spiked blanks (A) and the peak areas of the standards solution with a similar concentration as the post-spiked extracts (B) through the following equation: ME (%) = A/B x 100 [81, 84]. A value of 100% indicates the absence of matrix effect, the

ionization enhancement and the ionization suppression is given respectively by 100%, > 100% or < 100%.

3.7. Quantification in drinking waters

Several DW samples from different sources, namely tap water (n = 13), fountain water (n = 5) and well water (n = 5), were collected in the end of May 2015, from various locations of the north of Portugal and analyzed by the proposed method. Samples were stored at 4 °C until extraction, which was performed within 24 h. Prior to extraction sodium thiosulfate (1.5 mL of a solution 6 g L^{-1}) was added to each sample to reduce any residual chlorine that had been added as a disinfectant and the pH was adjusted to 3, with sulfuric acid.

3.8. Lab-scale photolysis (UV) and ozonation (O_3) experiments

Photolysis and ozonation experiments were carried out at lab-scale (*Figure 9*) during 30 min, in a 1 L reactor loaded with 750 mL of the tap water samples magnetic stirred at 350 rpm, collected from the water supply network of Porto (Portugal), to assess the removal of the target micropollutants.

Figure 9 - Photolysis (left) and ozonation (right) experiments at lab-scale.

Photolysis assays were performed using a Heraeus TNN 15/32 low-pressure mercury-vapour lamp (MVP), with an emission line at 253.7 nm (UVC - 3 W of radiant flux), located axially in the lab-scale reactor and held in a quartz immersion tube. For ozonation experiments, a

BMT 802X ozone generator was used to produce ozone from pure oxygen and the concentration of ozone was monitored with a BMT 964 ozone analyser. The constant ozone flow rate and inlet concentration were respectively 150 cm^3 min^{-1} and 50 g m^{-3} [85]. Gas washing bottles filled with potassium iodide solution were used to remove the ozone leaving the reactor in the gas phase. At the end of the ozonation assays, the gas stream was replaced by oxygen for 30 min, at the same flow rate (150 cm^3 min^{-1}), to remove the dissolved ozone.

4. Results and discussion

4.1. UHPLC-MS/MS

4.1.1. Chromatographic separation

Chromatographic separation was optimized using a Kinetex™ 1.7 µm XB-C18 100 Å column (100 × 2.1 mm, i.d.). The main advantages of this stationary phase are the low column length and diameter and sub-2 µm particles that allow short and high resolution chromatographic runs. Since the present work deals with different groups of compounds with a vast range of physical-chemical characteristics, the ideal mobile phase for part of the target compounds might lead to low sensitivity for many other analytes. In order to optimize a mobile phase for a wide set of analytes, the challenge consists on privileging the better results obtained for compounds with a general lower signal intensity. In this context, nine mobile phases were tested in order to improve resolution and enhance the sensitivity of the studied analytes, at isocratic mode with a flow rate of 0.20 mL min^{-1}. The proportion of organic and aqueous phases was 50/50 (v/v). While methanol, ethanol or acetonitrile were tested as organic phases, ultrapure water, 10 mM of ammonium acetate or 0.1% of formic acid were used to choose the best aqueous phase (*Figure 10*).

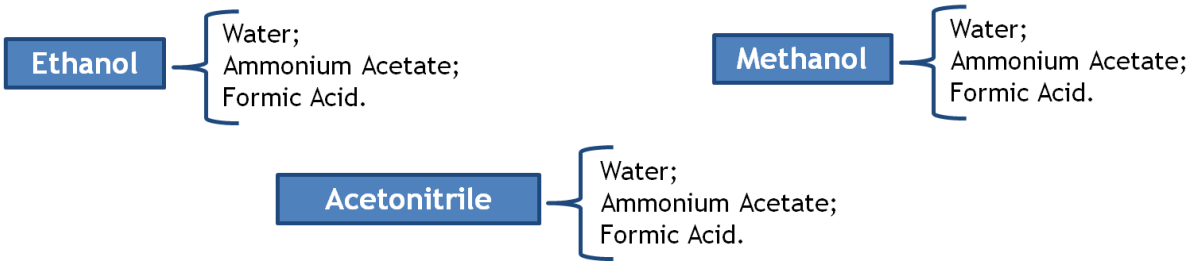


Figure 10 - Combinations of organic and aqueous phases tested.

The chromatograms obtained for a standard solution with the target compounds at 50 µg L^{-1}, showed that both ethanol and acetonitrile offered higher signal intensity than methanol. For most analytes, both additives ammonium acetate and formic acid did not enhance the peak shape and resolution, using either ethanol or acetonitrile. *Figures 18, 19 and 20*, present in *Appendix B*, shows the chromatograms obtained for fluoxetine.

The chosen mobile phase consisted in a mixture of ethanol and ultrapure water (70/30, v/v), considering the recent trends of green analytical chemistry [86] and the use of a UHPLC instrument that enables high pressure and the possibility to work with more viscous

solvents than the convectional ones. After this, several parameters were also optimized, namely the injection volume, the column oven temperature, the flow rate and the elution mode, aiming to improve resolution and peak shape and to get a short analysis time. The injection volume was set at 5 µL and the isocratic mode was selected with a flow rate of 0.20 mL min^{-1}, during 15 min. As raising the temperature reduces the viscosity of the mobile phase, the optimized oven temperature was 35 °C, improving the peak shape of the analytes and reducing the analysis time. The short run time and the low volume of a non-toxic organic phase as ethanol is a great improvement in the method development, comparing to chromatographic methods for DW analysis, using methanol [42] or acetonitrile [16, 71] as organic phases.

4.1.2. Mass spectrometry (MS/MS)

The tandem MS detection using a triple quadrupole enabled the simultaneous quantification of the 23 target analytes at trace levels and their identity confirmation. Precursor ion for each analyte was selected through the single direct injection of each target compound at 10000 µg L^{-1} in full scan mode, under both positive and negative modes. From all the compounds studied in this work, 18 compounds and 2 internal standards showed higher response under positive mode of ionization, with the protonated molecular ion of each compound $[M+H]^+$ chosen as precursor ion, whereas 6 substances (5 compounds and 1 internal standards) were more intense in negative ionization mode, using the deprotonated molecular ion of each compound $[M-H]^-$ as precursor ion.

Most compounds presented two or more SRM and the most abundant product ion from each precursor ion (SRM1) was selected for quantification (*Table 4*). Both the retention time of each analyte (*Table 5)* and the second most abundant transition (SRM2) were used for confirmation purpose of the identity of the compounds, through the ion ratio (SRM1/SRM2), according to European Commission Decision 2002/657/EC. Two pharmaceuticals and one pesticide (e.g. tramadol, fluoxetine and pentachlorophenol) demonstrated a poor fragmentation and only one SRM could be monitored, a drawback overcome by the internal standard calibration using surrogate standards. The optimized declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) for each SRM of each analyte, as well as the ion ratio are described in *Table 4.*

Table 4 - Optimized mass spectrometer parameters for SRM analysis of the target analytes.

Class and sub-class	Analyte	IS set[a]	ESI mode (NI[b] or PI[c])	Precursor ion (m/z)	Quantification (SRM1)				Identification (SRM2)				Ion ratio (±SD)
					Product Ion (m/z)	DP[d] (V)	CE[e] (V)	CXP[f] (V)	Product Ion (m/z)	DP[d] (V)	CE[e] (V)	CXP[f] (V)	
Pharmaceuticals													
Anti-inflammatories	Diclofenac [h]	1	NI	294.1	250.10	14	12	17	214.05	14	21	23	19.69 (±0.09)
	Tramadol	2	PI	264.0	57.70	-30	-25	-30	-	-	-	-	n.a.
	Ketoprofen-d3 (1)		NI	256.2	212.10	12	8	22	-	-	-	-	n.a.
Antibiotics	Azithromycin [h]	2	PI	749.5	83.15	-36	-52	-13	116.10	-36	-47	-21	1.14 (±0.11)
	Clarithromycin [h]	2	PI	748.4	158.15	-40	-30	-15	590.30	-40	-21	-28	3.22 (±0.07)
	Trimethoprim	2	PI	290.5	230.00	-30	-24	-24	123.05	-30	-26	-21	1.26 (±0.09)
Anticoagulant	Warfarin	2	PI	309.0	163.00	-15	-16	-28	251.05	-15	-21	-26	1.32 (±0.10)
Antiplatelet agent	Clopidogrel	2	PI	321.6	212.05	-27	-17	-22	184.00	-27	-23	-17	1.67 (±0.07)
Beta-blockers	Metoprolol	2	PI	267.8	116.15	-20	-20	-20	74.15	-20	-23	-28	1.18 (±0.06)
Lipid regulators and cholesterol lowering statin drugs	Atorvastatin	2	NI	557.3	278.20	20	47	30	397.25	20	30	27	2.24 (±0.28)
	Bezafibrate	2	NI	360.2	274.15	17	17	19	154.05	17	31	29	1.77 (±0.19)
Psychiatric drugs	Carbamazepine	2	PI	236.9	194.10	-28	-20	-19	192.10	-28	-22	-19	4.22 (±0.18)
	Citalopram	2	PI	324.5	109.10	-24	-27	-19	262.00	-24	-20	-27	4.22 (±0.18)
	Venlafaxine	2	PI	277.8	58.10	-30	-22	-22	260.15	-30	-12	-27	2.90 (±0.11)
	Fluoxetine	2	PI	310.0	44.15	-15	-14	-16	-	-	-	-	n.a.
	Fluoxetine-d5 (2)		PI	315.0	44.15	-16	-14	-15	-	-	-	-	n.a.
Metabolite	Norfluoxetine	2	PI	296.0	134.15	-30	-8	-13	30.25	-30	-13	-30	1.52 (±0.04)

Table 4 – Continued.

Class and sub class	Analyte	IS set[a]	ESI mode (NI[b] or PI[c])	Precursor ion (m/z)	Quantification (SRM1)				Identification (SRM2)				Ion ratio (±SD)
					Product Ion (m/z)	DP[d] (V)	CE[e] (V)	CXP[f] (V)	Product Ion (m/z)	DP[d] (V)	CE[e] (V)	CXP[f] (V)	
Pesticides													
Chloroacetanilide	Alachlor [i]	3	PI	270.0	238.10	-13	-11	-24	162.05	-13	-20	-15	2.07 (±0.09)
Triazine	Atrazine [i]	3	PI	215.9	174.05	-23	-18	-30	68.15	-23	-37	-24	2.44 (±0.10)
	Simazine [i]	3	PI	201.9	124.10	-22	-18	-11	131.95	-22	-20	-23	1.35 (±0.20)
	Atrazine-d5 (3)		PI	221.0	179.05	-11	-19	-18	-	-	-	-	n.a.
Organophosphorus	Chlorfenvinphos [i]	3	PI	360.5	155.10	-25	-40	-16	99.10	-25	-15	-15	1.49 (±0.14)
	Isoproturon [i]	3	PI	206.9	72.10	-22	-21	-27	46.15	-22	-18	-16	2.19 (±0.07)
Organochlorine	Pentachlorophenol [i]	3	PI	265.1	35.15	13	48	30	-	-	-	-	n.a.
Herbicide	Clofibric acid	3	NI	213.1	127.00	10	13	13	85.00	10	11	13	8.42 (±0.31)
Industrial compound	Perfluorooctanesulfonic acid [i]	3	NI	498.7	79.95	18	50	14	99.00	18	46	18	3.15 (±0.13)

[a] IS is internal standard.

[b] NI is negative ionization mode.

[c] PI is positive ionization mode.

[d] DP is the declustering potential.

[e] CE is the collision energy.

[f] CXP is the collision cell exit potential.

[g] n.a. is not applicable.

[h] Included in the watch list for the intent prioritization process at European Union level (Annex of the EU Decision 2015/495).

[i] PSs of the Directive 2013/39/EU.

Table 5 - Retention time, range, linearity, instrument and method detection and quantification limits for each target analyte.

Class and sub-class	Analyte	Retention time (min)	Range (ng L^{-1})	r^2	IDL[a] (µg L^{-1})	IQL[b] (µg L^{-1})	MDL[c] (ng L^{-1})	MQL[d] (ng L^{-1})
Pharmaceuticals								
Anti-inflammatories	Diclofenac	1.27	0.75-40	0.9982	0.21	0.62	0.17	0.52
	Tramadol	5.65	0.75-40	0.9976	0.09	0.27	0.07	0.22
Antibiotics	Azithromycin	8.08	0.75-40	0.9969	0.24	0.74	0.20	0.61
	Clarithromycin	8.47	0.75-40	0.9957	0.13	0.39	0.11	0.32
	Trimethoprim	4.00	0.75-40	0.9993	0.08	0.25	0.07	0.21
Anticoagulant	Warfarin	1.28	0.75-40	0.9965	0.21	0.63	0.17	0.52
Antiplatelet agent	Clopidogrel	2.11	0.75-40	0.9982	0.01	0.04	0.01	0.04
Beta-blockers	Metoprolol	6.29	0.75-40	0.9984	0.06	0.18	0.05	0.15
Lipid regulators and cholesterol lowering statin drugs	Atorvastatin	1.21	0.75-40	0.9971	0.09	0.27	0.07	0.23
	Bezafibrate	1.18	0.75-40	0.9917	0.23	0.69	0.19	0.57
Psychiatric drugs	Carbamazepine	1.32	0.75-40	0.9966	0.23	0.71	0.19	0.59
	Citalopram	6.06	0.75-40	0.9961	0.11	0.32	0.09	0.26
	Venlafaxine	6.84	0.75-40	0.9978	0.12	0.38	0.10	0.32
	Fluoxetine	8.86	0.75-40	0.9963	0.05	0.16	0.04	0.13
Metabolite	Norfluoxetine	8.93	0.75-40	0.9975	0.06	0.19	0.05	0.16
Pesticides								
Chloroacetanilide	Alachlor	1.65	0.75-40	0.9975	0.11	0.34	0.09	0.28
Triazine	Atrazine	1.33	0.75-40	0.9945	0.14	0.44	0.12	0.37
	Simazine	1.21	0.75-40	0.9983	0.18	0.55	0.15	0.46
Organophosphorus	Chlorfenvinphos	1.62	0.75-40	0.9971	0.21	0.65	0.18	0.54
Phenylurea	Isoproturon	1.34	0.75-40	0.9968	0.05	0.14	0.04	0.12
Organochlorine	Pentachlorophenol	1.55	0.75-40	0.9986	0.24	0.72	0.20	0.60
Herbicide	Clofibric acid	1.23	0.75-40	0.9995	0.16	0.50	0.14	0.42
Industrial compound	PFOS	1.07	0.75-40	0.9957	0.08	0.23	0.06	0.19

For the optimization of the desolvation and source temperatures, nebulizing and drying gas flows and capillary voltage, a working standard solution with the target compounds at 50 µg L^{-1} was injected and the peak areas were compared. The collision induced dissociation gas (CID) used was argon at 230 kPa. The results obtained are presented in *Appendix C* in *Figures 21, 22, 23, 24* and *25*. By the analyses of the results, the best conditions for these MS parameters were: 2.5 L min^{-1} for nebulizing gas flow, 10 L min^{-1} for drying gas flow, 0.5 kV for capillary voltage, 450 ºC for source temperature and 200 ºC for desolvation temperature.

4.2. SPE optimization

A solid phase extraction (SPE) procedure was developed for the pre-concentration and posterior analysis by UHPLC-MS/MS of several pharmaceutical compounds, a pharmaceutical metabolite, pesticides and one industrial compound in DW. A detailed optimization study was carried out on the most relevant parameters that affect recovery rates and matrix effects, namely the type of cartridges, the sample pH, the extraction solvents, the sample volume and the addition of quelating and dechlorination additives. These parameters and the respective results obtained are described below.

- **Sample pH**

Preliminary studies were performed to evaluate the performance of different sample pH, by extracting 250 mL of tap water samples through Oasis® HLB (Hydrophilic-Lipophilic-Balanced) cartridges. The water samples were adjusted to different pH (3, 7 and 9) and tested using a conventional solvent, i.e. methanol. The results are presented below (*Figures 11 a) and b)*).

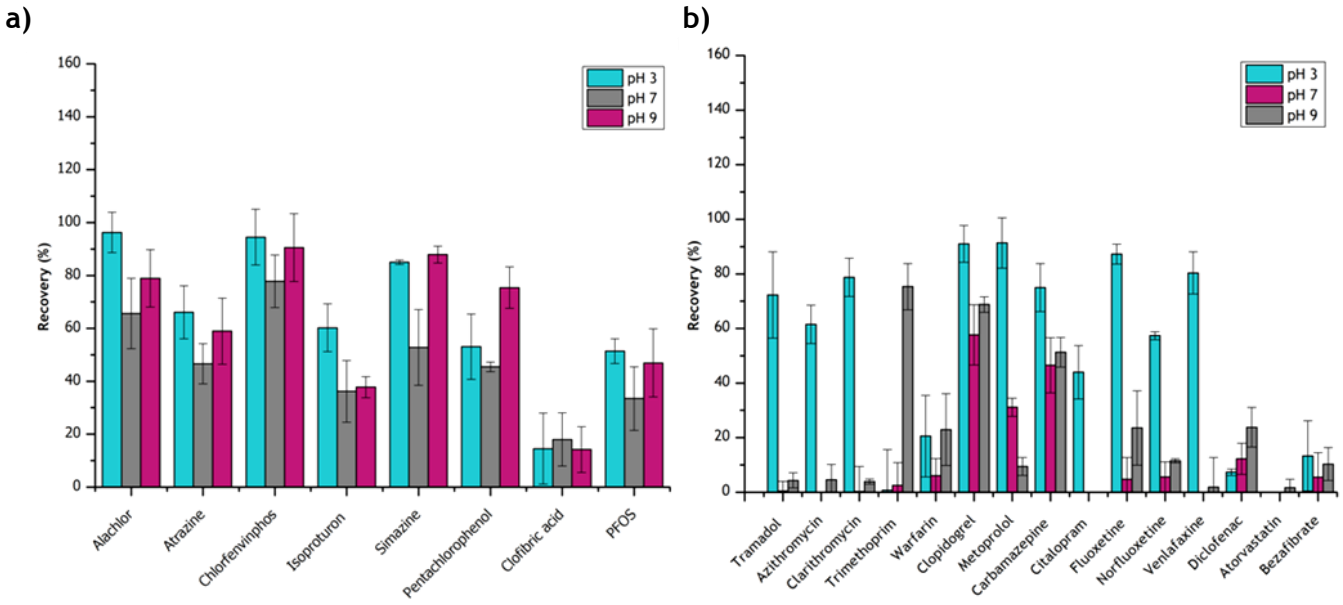


Figure 11 - Recoveries obtained for micropollutants for different pH (3, 7 and 9), extracting 250 mL of tap water samples through Oasis® HLB cartridges and using methanol as solvent: a) pesticides and industrial compound; b) pharmaceuticals and metabolite.

Figures 11 a) and b) show that the acid pH provided higher recoveries for the majority of the compounds, and in particular for pharmaceuticals. Since the pH had a great effect on the recoveries, depending on the compounds, a compromise should be attained to allow including as much analytes as possible. Thus, the selected sample pH was 3.

- **Extraction Solvent**

Afterwards, Oasis® HLB cartridges were employed to extract 250 mL of tap water samples at pH 3 (optimized for methanol), but in this case using ethanol as conditioning and elution solvent, due to the known toxicity of methanol (usually used for SPE).The results are shown in *Figures 12 a) and b)*.

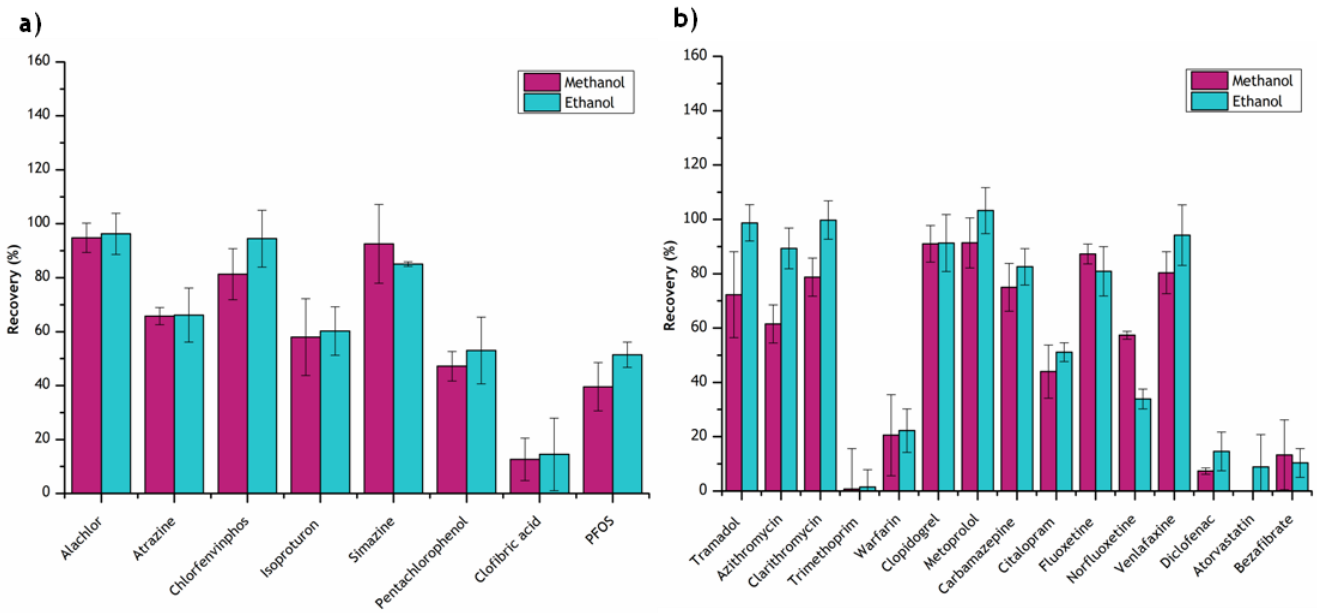


Figure 12 - Recoveries obtained for micropollutants for different solvents (methanol and ethanol), extracting 250 mL of tap water samples (pH 3) through Oasis® HLB: a) pesticides and industrial compound; b) pharmaceuticals and metabolite.

Comparing the results presented in *Figure 12*, it was possible to select the solvent that showed the best recoveries. Ethanol allows to obtain recoveries slightly higher than methanol for the vast of compounds. Moreover, ethanol is considered a "green" solvent, i.e., minimizes the environmental impact resulting from the use of solvents, and follows the guidelines of green analytical chemistry [86, 87]. In fact several methods reported in the literature employ solvents such as methanol or acetonitrile, presenting high toxicity [6, 16, 42, 48, 71, 73]; thus, the use of ethanol is an important progress in the present work.

- **Type of cartridges**

After optimizing the pH and the solvent for SPE using the versatile Oasis® HLB cartridges, which are suitable for most compounds (acidic, basic and neutrals), the performance of two other different cartridges was evaluated: Oasis® MCX useful for extraction of basic compounds and Oasis® MAX adequate for extraction of acidic compounds. *Figure 13* shows the results obtained for pesticides and the industrial compound *Figure 13 a*) and pharmaceutical compounds and metabolite *Figure 13 b*), respectively.

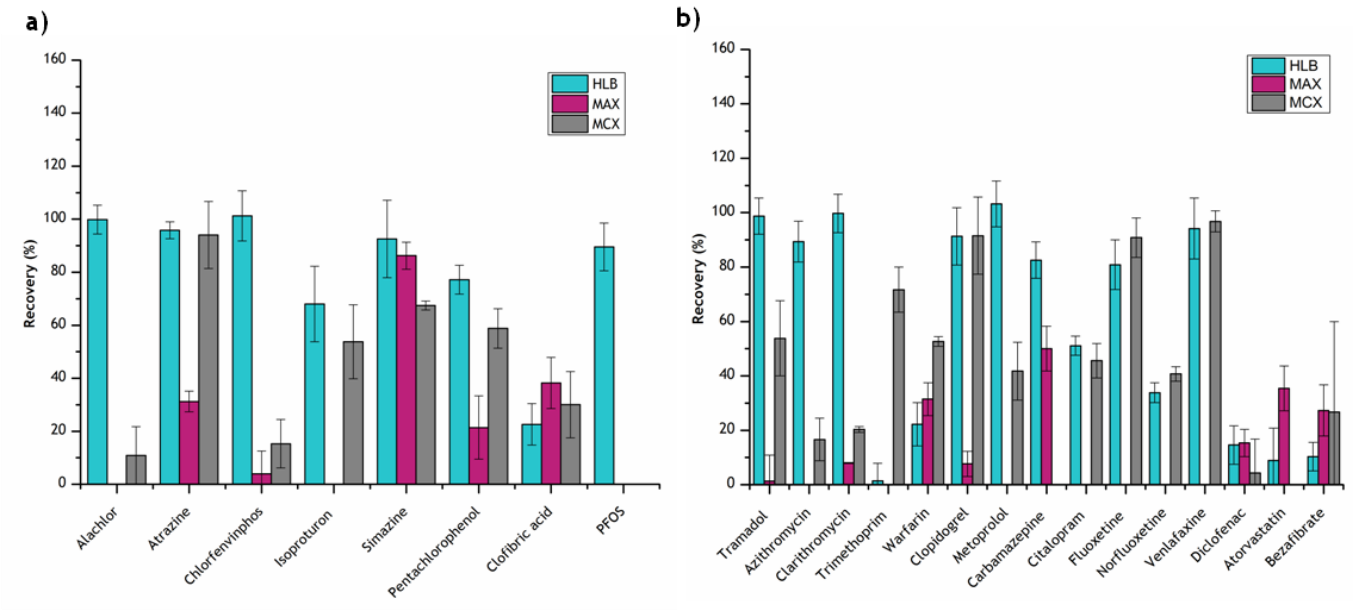


Figure 13 - Recoveries obtained for micropollutants for different cartridges (Oasis® HLB, MAX and MCX) extracting 250 mL of tap water samples (pH 3 for HLB and MCX; pH 9 for MAX) and using ethanol (HLB) or methanol (MAX and MCX) as solvents: a) Pesticides and industrial compound; b) Pharmaceuticals and metabolite.

By the results shown in *Figure 13 a)*, it was possible to verify that the Oasis® HLB cartridges provided higher recoveries for most compounds. *Figure 13 b)* shows that the class of antidepressants (citalopram, fluoxetine, its metabolite norfluoxetine and venlafaxine) and the trimethoprim had a good recovery using Oasis® MCX. These results were expected, due to the high p*K*a of these compounds, which is in the range of 9. Clofibric acid, diclofenac, atorvastatin and bezafibrate had a higher recovery when extracted by MAX cartridges, owing to their acidic nature (p*K*a values approximately 4). Since, in this work the target analytes have different physical-chemical characteristics, Oasis® HLB was the adsorbent that gave better recoveries, in general, as observed in previous studies conducted by Maldaner *et al*. [48] and Gros *et al*. [6]. Therefore, Oasis® HLB was selected for subsequent recovery experiments.

- **Sample volume**

Different sample volumes were tested (100, 250, 500 and 1000 mL) to determine the breakthrough volume, the higher volume that allows the maximum extraction efficiency and from which extraction efficiency declined [88]. *Figures 14 a)* and *b)* show the results obtained for the studied compounds.

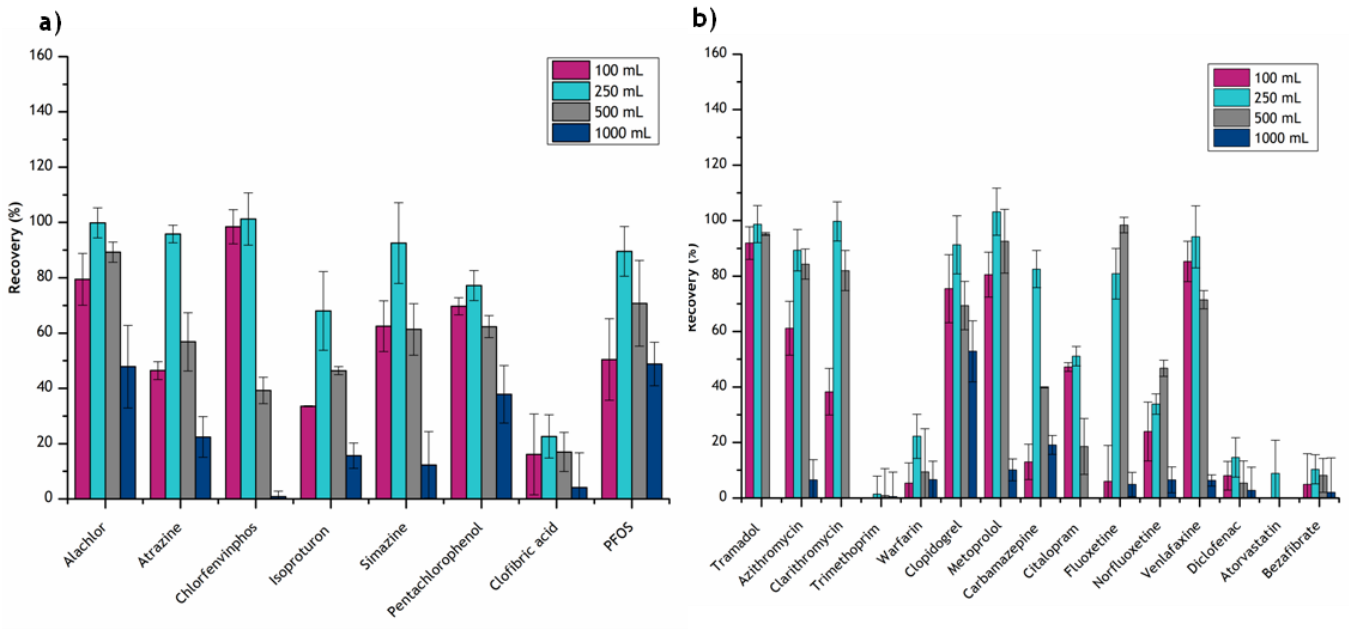


Figure 14 – Recoveries obtained for micropollutants, extracting different sample volumes (100, 250, 500 and 1000 mL), of tap water samples (pH 3) through Oasis® HLB cartridges, using ethanol as a solvent: a) pesticides and industrial compound; b) pharmaceuticals and metabolite.

The sample volume of 250 mL provided the highest recoveries for the majority of the compounds, except for fluoxetine and norfluoxetine, being selected as the optimized sample volume. Although a higher volume would give a theoretical higher enrichment factor, the results showed that recovery rates decreased using higher sample volumes, for most compounds, due to the referred above phenomenon of decrease of extraction efficiency above the so-called breakthrough volume, as previously observed by Bielicka-Daszkiewicz *et al.* [89] and Ribeiro *et al.* [90].

- **Quelating and dechlorination agents**

Subsequently, the quelating and dechlorination effect were studied. Whilst a solution of EDTA was added to the water samples to test the quelating effect, acid ascorbic or sodium thiosulfate were added to the water samples to assess the dechlorination effect (*Figures 15 a)* and *b)*.

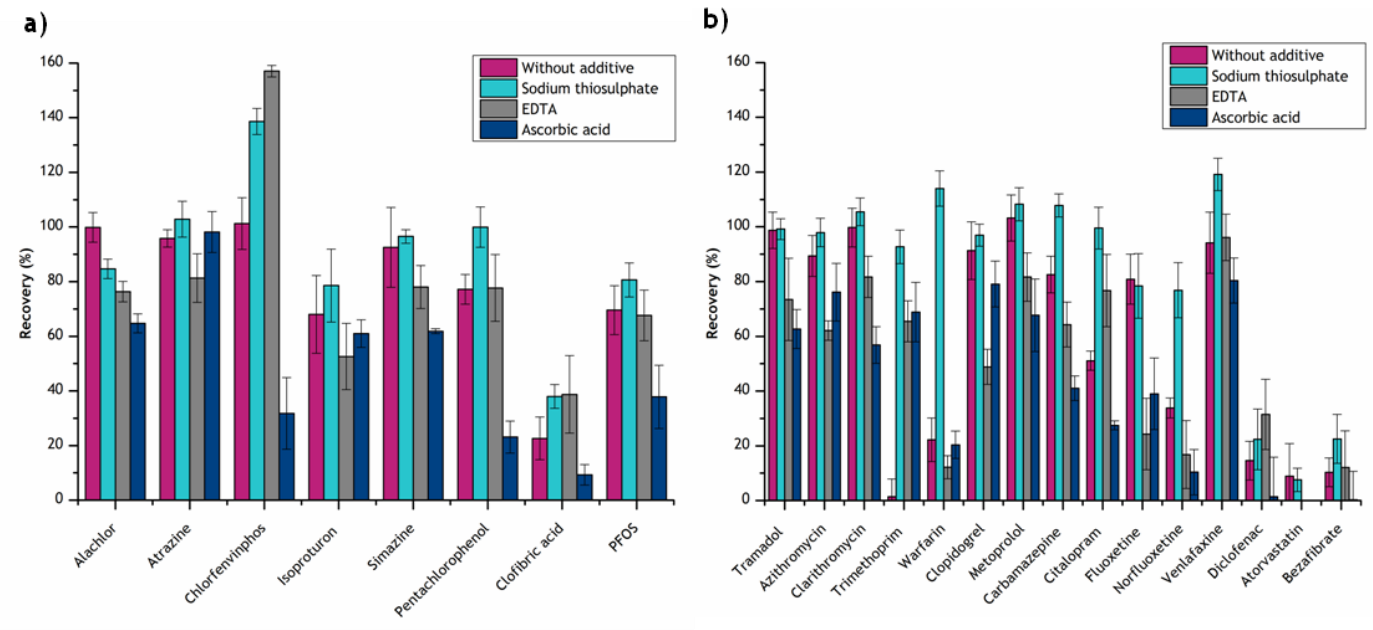


Figure 15 – Recoveries obtained for micropollutants extracting 250 mL of tap water samples (pH 3), with the addition of a quelating (EDTA) or dechlorination additives (sodium thiosulphate or ascorbic acid) through Oasis® HLB cartridges, using ethanol as a solvent: a) pesticides and industrial compound; b) pharmaceuticals and metabolite.

Regarding to the addition of EDTA, it was possible to verify that it improved the extraction efficiency of only four compounds in a great extent, compared with the results obtained for samples without additive, namely for chlorfenvinphos, clofibric acid, trimethoprim and diclofenac. This could be explained by the fact that these compounds might bind to residual metals present in the sample matrix, resulting in low extraction recoveries [6]. By adding EDTA, soluble metals bind to the quelating agent, increasing the extraction efficiency of some compounds that are available to be extracted and detected [6]. This phenomenon was previously observed by several authors [6, 42, 73, 91].

Concerning the dechlorination agents, the results showed that sodium thiosulfate gave better results than ascorbic acid. The addition of sodium thiosulfate increased the extraction recoveries, probably because it reduced the residual chlorine that had been added as a disinfectant in the DW supply [16]. Therefore, sodium thiosulfate was selected for subsequent SPE experiments. Beyond the experiments described above, the effects of filtering and aeration of the water samples and the simultaneous addition of EDTA and sodium thiosulfate were also studied. However, the recovery efficiency was not improved (data not shown).

The main objective of the optimization of the sample preparation methodology was the development of a single SPE procedure, allowing the extraction of a large group of compounds with different physical-chemical characteristics. As a result, and according to the higher recoveries obtained for most of the target compounds, the selected conditions

were: Oasis® HLB cartridges, sample pH 3, ethanol as solvent, 250 mL of water samples and 1.5 mL of sodium thiosulfate (6 g L^{-1}) as dechlorination agent.

- **Cartridges reuse**

Finally, the reuse of the cartridges was evaluated for 3 consecutive days. The recoveries obtained for each procedure are shown in the following figure (*Figures 16 a)* and *b)*).

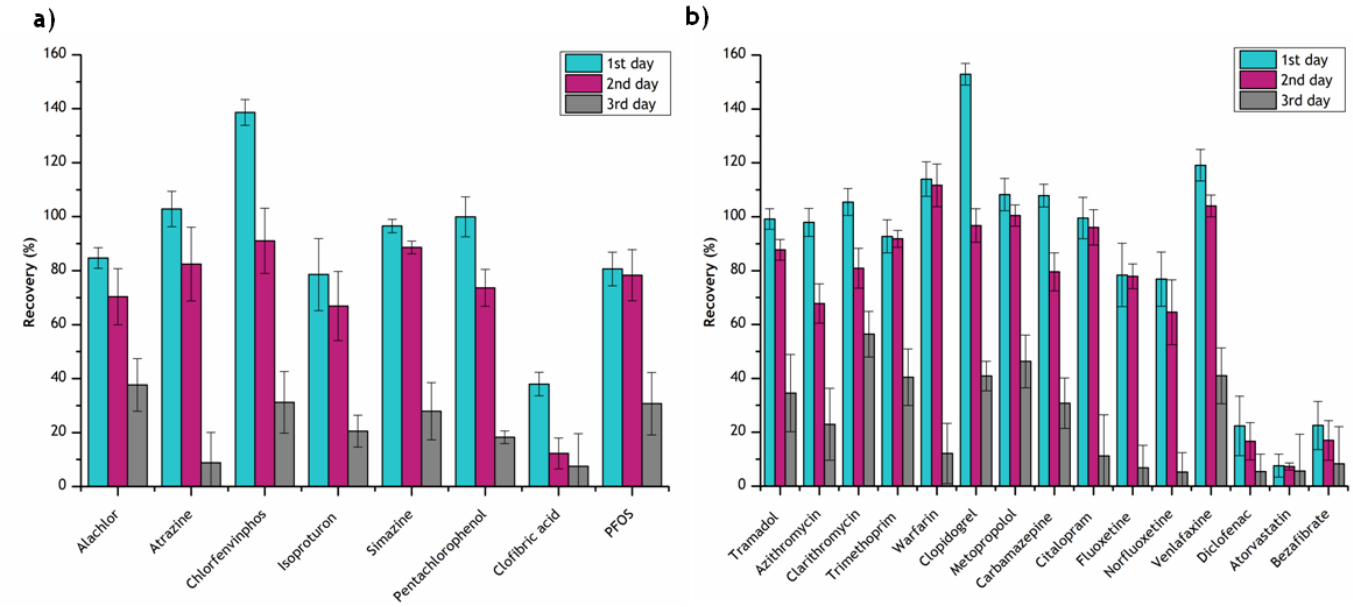


Figure 16 - Recoveries obtained for micropollutants extracting 250 mL of tap water samples (pH 3), with the addition of sodium thiosulphate (6 g L^{-1}), using ethanol as a solvent through new and twice reused Oasis® HLB cartridges: a) pesticides and industrial compound; b) pharmaceuticals and metabolite.

It was possible to verify (*Figure 16)* that each reuse led to a loss of capacity of retention for the cartridges, reflected by the decrease of the recovery of the compounds. The first reuse of the cartridges still allows achieving good recoveries for most analytes. In average, efficiency decreased by 14%. Comparing the results obtained in the second reuse with the results obtained in first reuse, it was possible to verify that the loss was higher, approximately 50%. Here, it was demonstrated that reusing cartridges is not a good practice for analytical purposes that require a high reproducibility.

4.3. Method validation

The SPE-UHPLC-MS/MS method was validated according to international criteria [78] and work published elsewhere [79, 81, 83]. Recovery, accuracy, intra and inter-batch precision values are described in *Table 6*.

Table 6 - Recovery, accuracy, precision (intra- and inter-batch) and matrix effect for each target analyte.

Class and sub-class	Analyte	Recovery (%)	Accuracy (%)	Intra-batch precision RSD (%)	Inter-batch precision RSD (%)	Matrix effect (%)
Pharmaceuticals						
Anti-inflammatories	Diclofenac	22.4 ± 11.1	108	1.67 - 8.48	10.1	22.2 ± 2.28
	Tramadol	99.2 ± 3.82	102	2.28 - 3.55	12.9	117 ± 0.09
Antibiotics	Azithromycin	97.9 ± 5.19	89.2	7.93 - 9.75	9.38	23.7 ± 8.36
	Clarithromycin	106 ± 5.04	99.9	7.75 - 10.0	11.2	26.4 ± 11.5
	Trimethoprim	92.7 ± 6.14	97.1	2.99 - 5.80	7.21	64.9 ± 13.3
Anticoagulant	Warfarin	114 ± 6.43	118	7.67 - 15.2	10.6	193 ± 1.74
Antiplatelet agent	Clopidogrel	96.9 ± 10.0	99.2	2.75 - 8.24	6.89	77.4 ± 10.3
Beta-blockers	Metoprolol	103 ± 8.47	96.5	3.26 - 14.0	13.2	113 ± 5.58
Lipid regulators and cholesterol lowering statin drugs	Atorvastatin	7.57 ± 4.25	91.4	8.73 - 14.0	11.0	13.1 ± 3.22
	Bezafibrate	22.5 ± 8.95	92.3	7.55 - 11.3	9.71	6.18 ± 5.22
Psychiatric drugs	Carbamazepine	108 ± 4.22	101	9.76 - 15.0	8.38	30.4 ± 8.39
	Citalopram	99.5 ± 7.67	86.6	5.09 - 11.4	14.5	113 ± 11.7
	Venlafaxine	119 ± 5.87	113	1.11 - 4.60	14.5	109 ± 2.11
	Fluoxetine	78.4 ± 11.8	92.5	0.77 - 3.96	5.19	95.2 ± 6.36
Metabolite	Norfluoxetine	76.9 ± 10.1	102	3.04 - 6.79	6.99	95.2 ± 4.36
Pesticides						
Chloroacetanilide	Alachlor	84.7 ± 3.57	102	6.39 - 14.9	8.97	99.2 ± 10.3
Triazine	Atrazine	103 ± 6.57	92.3	2.60 - 6.47	7.86	52.5 ± 15.4
	Simazine	96.5 ± 2.49	80.1	3.86 - 9.23	8.35	49.8 ± 2.41
Organophosphorus	Chlorfenvinphos	139 ± 4.78	98.6	5.01 - 14.7	14.8	96.9 ± 2.04
Phenylurea	Isoproturon	78.6 ± 13.3	99.2	2.00 - 4.10	5.02	34.5 ± 9.43
Organochlorine	Pentachlorophenol	99.9 ± 7.40	94.1	7.75 - 13.2	8.65	57.5 ± 9.03
Herbicide	Clofibric acid	38.0 ± 4.38	92.7	6.20 - 11.0	6.57	19.1 ± 6.46
Industrial compound	PFOS	80.6 ± 6.24	89.0	5.30 - 13.5	4.51	48.7 ± 1.37

The recovery of the target analytes was compared, after pre-concentration of blank samples and 35 ng L^{-1} spiked samples, using the optimized SPE procedure (*Section 4.2. SPE optimization*). The recoveries evaluated for the DW matrix were between 7.57 (± 4.25)% and 139 (± 4.78)% (*Table 6*). Peak areas of the target analytes found in the DW blank matrix were deducted for recovery rate estimation. The dissimilar recoveries are owing to the wide chemistry nature of the target compounds and were taken into account using the matrix match calibration curves and internal standards addition before SPE. Three QC standard extracts were used to evaluate the accuracy and intra and inter-batch precision. The accuracy ranged from 86.6% to 118% (*Table 6*), which is within the range of +/- 20% of the nominal concentration, according to the international guidelines (80—120%) [78]. Relative standard deviation (RSD) of the replicate analyses was used to express the precision of the method (*Table 6*). The SPE-UHPLC-MS/MS is precise between the different QC of the same concentration (intra-batch < 15.2% and inter-batch < 14.8%), meeting the international criteria which suggest an agreement of the results traduced by a RSD lower than 15% (or 20% for the lower concentration QC) [78]. The calibration curves (*Table 5*) were generated using the internal calibration method through spiking samples with isotopically labeled internal standards, before SPE extraction. Diverse sets of compounds were defined to relate with each internal standard (*Table 4*), depending on the acid-basic nature, as other published works dealing with multi-class determination [6, 42], since labelled standards for all compounds are not available. The injection of the reconstituted ethanolic extracts gave coefficients of determination between 0.9917—0.9995 in the range of 0.75—40 ng L^{-1} for all compounds. The ranges of method detection and quantification limits were respectively 0.01—0.20 ng L^{-1} and 0.04—0.61 ng L^{-1}, allowing to detect the target contaminants at residual concentrations.

4.4. Matrix effects

The matrix effect was determined to assess the influence of the matrix in the ionization process occurring in the ionization source of the mass spectrometer. The ionization of the analytes can be enhanced or reduced, depending on the matrix. This effect was estimated by the post-extraction addition method, which consists in the comparison of chromatograms of SPE extracts of blank samples spiked with a solution containing the target compounds (post-spiked blank extracts), with chromatograms of the standard solution with the theoretical concentration of the extracts. The percentage ratio between the post-spiked blank extracts and ethanolic standard solutions, were between

6.18 (± 5.22)% and 193.43 (± 1.74)%. Most compounds presented signal suppression, i.e. matrix effect < 100%, namely diclofenac, azithromycin, clarithromycin, trimethoprim, clopidogrel, atorvastatin, bezafibrate, carbamazepine, atrazine, simazine, isoproturon, pentachlorophenol, clofibric acid and PFOS (*Table 6*). Tramadol, metoprolol, citalopram and venlafaxine had a slight ionization enhancement (matrix effect > 100%) while the signal of warfarin was highly increased. Compounds with almost no matrix effect, under the conditions of the current work, were fluoxetine, norfluoxetine, alachlor and chlorfenvinphos.

4.5. Quantification of micropollutants in drinking waters

DW samples collected in the end of the May 2015, from various locations of the north of Portugal and from different sources, namely tap water (n = 13), fountain water (n = 5) and well water (n = 5), were analysed using the optimized SPE-UHPLC-MS/MS method referred above. The results obtained are summarized in *Table 7*.

Table 7 - Concentrations of micropollutants (ng L^{-1}) detected in tap, fountain and well water samples analyzed.

Class and sub-class	Analyte	Tap water (n=13)		Fountain water (n= 5)		Well water (n=5)	
Pharmaceuticals		**Concentration (ng L^{-1})**	**Frequency**	**Concentration (ng L^{-1})**	**Frequency**	**Concentration (ng L^{-1})**	**Frequency**
Anti-inflammatories	Diclofenac	<MQL - 7.87	7/13	3.95 - 7.66	4/5	1.60 - 36.19	5/5
	Tramadol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Antibiotics	Azithromycin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Clarithromycin	< MQL	1/13	n.d.	n.d.	1.14	1/5
	Trimethoprim	< MQL	1/13	< MQL	1/5	0.86	1/5
Anticoagulant	Warfarin	0.39 - 3.89	5/13	4.07	1/5	11.23	1/5
Antiplatelet agent	Clopidogrel	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beta-blockers	Metoprolol	< MQL	5/13	n.d.	n.d.	< MQL	1/5
Lipid regulators and cholesterol lowering statin	Atorvastatin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Bezafibrate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Psychiatric drugs	Carbamazepine	3.34	1/13	n.d.	n.d.	58.82	1/5
	Citalopram	< MQL	1/13	n.d.	n.d.	n.d.	n.d.
	Venlafaxine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Fluoxetine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Metabolite	Norfluoxetine	< MQL	13/13	< MQL	1/5	< MQL	1/5
Pesticides							
Chloroacetanilide	Alachlor	< MQL	4/13	n.d.	n.d.	3.07	1/5
Triazine	Atrazine	1.14 - 2.24	6/13	1.59 - 103.22	3/5	1.66	1/5
	Simazine	< MQL - 1.45	4/13	< MQL - 2.20	2/5	2.84 - 28.36	2/5
Organophosphorus	Chlorfenvinphos	2.46 - 6.50	2/13	0.49-3.89	2/5	n.d.	n.d.
Phenylurea	Isoproturon	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Organochlorine	Pentachlorophenol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Herbicide	Clofibric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Industrial compound	PFOS	< MQL	1/13	n.d.	n.d.	11.72	1/5

From the 23 investigated compounds, 13 were detected in DW samples. Regarding tap water, 7 pharmaceuticals, 1 metabolite, 4 pesticides and 1 industrial compound were found in DW samples. Diclofenac, warfarin, norfluoxetine, atrazine and simazine were the most frequently compounds detected. The micropollutants found at higher concentrations were diclofenac and chlorfenvinphos, at 7.87 ng L^{-1} and 6.50 ng L^{-1}, respectively. The first is an anti-inflammatory included in the first watch list by the EU Decision 2015/495 and the pesticide chlorfenvinphos is considered PS in the Directive 2013/39/EU. Clarithromycin, trimethoprim, metoprolol, citalopram, norfluoxetine, alachlor and PFOS were detected under their MQL.

Concerning fountain water samples, 3 pharmaceutical compounds, 1 metabolite and 3 pesticides were detected. Diclofenac and atrazine were the most common micropollutants found in the fountain water samples analyzed. Trimethoprim and norfluoxetine were detected under their MQL. The compounds found at higher concentrations were diclofenac, included in the first watch list by the EU Decision 2015/495, and the pesticide atrazine, included in the Directive 2013/39/EU as a PS.

The results obtained for well water samples show that 11 micropollutants were found, namely 6 pharmaceuticals, 1 metabolite, 3 pesticides and 1 industrial compound. The anti-inflammatory diclofenac, was detected in all the samples at concentrations levels ranging from 1.6 to 36.19 ng L^{-1}. Metoprolol and norfluoxetine were detected under their MQL. The micropollutants found at higher concentrations were the pharmaceuticals diclofenac and carbamazepine and the pesticide simazine. The last one is also considered a PS in the Directive 2013/39/EU.

The analysis of the overall results for the tap, fountain and well water samples allow to verify that the most common micropollutants detected in these sources were diclofenac, trimethoprim, warfarin, norfluoxetine, atrazine and simazine.

The comparison of the results obtained in this work with similar studies conducted by several authors is difficult, since the consumption of pharmaceutical compounds, the intensity of agricultural and industrial activities varies among different regions. However, by the analysis of some studies reported in the literature, it was possible to verify that from the micropollutants under study in this work, carbamazepine is the compound most found up to 14.0 ng L^{-1} [6, 16, 42, 73]. Other compounds such as atenolol, clofibric acid, azithromycin, erythromycin, fluoxetine, diclofenac and atorvastatin were also detected but at very low levels [6, 48, 71-73].

4.6. Quantification of micropollutants in DW after photolysis (UV) and ozonation (O_3)

Tap water samples collected from the water supply network of Porto (Portugal), were spiked with the stock solution of micropollutants at 30 ng L^{-1} and submitted to UV photolysis or ozonation to assess the removal of the target micropollutants (*Figure 17*).

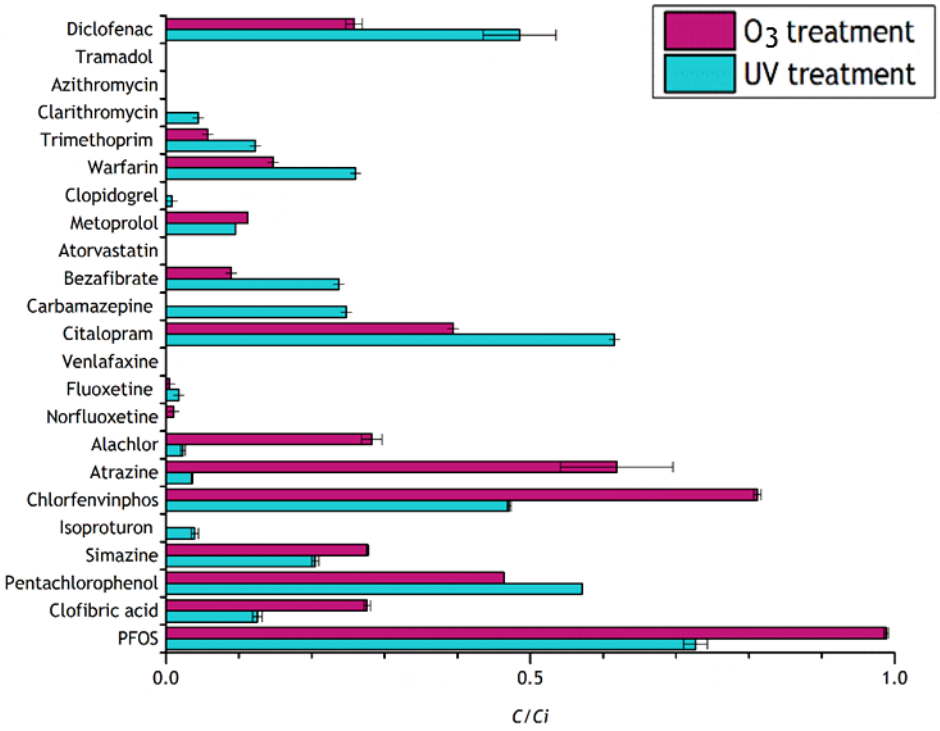


Figure 17 - Normalized concentration of the micropollutants (C/C_i) in DW, where C_i refers to the concentration before and C to that after the lab-scale UV photolysis or ozonation treatments.

The overall results (*Figure 17)* showed that, in general, pharmaceuticals were better removed by both treatments and that UV photolysis was more effective than ozonation for the pesticides and for the industrial compound. The opposite behavior was observed for pharmaceuticals, with a superior degradation by ozonation. It was possible to verify that 4 pharmaceutical compounds were completely removed with ozonation and UV treatment, namely tramadol, azithromycin, atorvastatin and venlafaxine. Azithromycin was recently included in the first watch list by the EU Decision 2015/495. Ozonation was already studied in a DWTP for metoprolol degradation, an incomplete removal being found. Concerning diclofenac and the antibiotic clarithromycin included in that list, diclofenac presented a removal average of ca. 52% and 74% for UV photolysis and ozonation treatments, respectively, while clarithromycin was highly removed by UV photolysis (96%) and

completely removed by ozonation. Other works have already reported removal rates of diclofenac from 97% to 100%, by ozonation performed at lab scale [74, 75].

Whilst clopidrogel, carbamazepine and the PS isoproturon were completely removed from water by lab-scale ozonation, the metabolite norfluoxetine was completely removed only by UV photolysis. Carbamazepine was already reported as persistent in a finished DW, after ozonation at pilot scale using a concentration of ozone lower than that used in the present thesis [76]. Regarding the other target pesticides considered as PSs in the Directive 2013/39/EU, simazine showed similar results for both treatments (removal > 70%). Alachlor and atrazine were highly removed by the UV photolysis (removal > 96%), with a lower removal rate observed in the ozonation experiments. Clofibric acid was also higher degraded by UV photolysis. Atrazine and clofibric acid were already reported as recalcitrant to ozonation in some studies conducted by Ternes *et al*. [75] and Hua *et al*. [76], on agreement with the results obtained in the present work. Chlorfenvinphos presented a removal average of ca. 53% and 19% for UV photolysis and ozonation treatments, respectively. Finally, pentachlorophenol showed a slightly higher percentage of removal by ozonation. The industrial compound PFOS, also included in the Directive 2013/39/EU as PSs, was poorly removed by both treatments, UV photolysis showing a better performance (removal average of ca. 27%).

According to the literature (*Table 2 of Section 2)*, ozonation is one of the most used treatments for the removal of micropollutants in DW facilities, and seen as one of the most efficient processes for the elimination of these compounds [63, 74, 75]. Removals higher than 90% were reached for several compounds, such as pesticides, anti-inflammatories, anti-epileptics and antibiotics. However, some substances seem to be recalcitrant by this process, namely clofibric acid, carbamazepine, atrazine, metoprolol and atenolol [75-77].

Studies on UV photolysis applied for tap water treatment are scarce, making difficult to compare the results obtained with this type of treatment.

5. Conclusions

A multi-residue green analytical methodology of SPE-UHPLC-MS/MS was optimized and validated to assess the occurrence of twenty three micropollutants in DW, including PSs of the European Directive 2013/39/EU (pesticides and one industrial compound) and substances of the first watch list of EU Commission Decision 2015/495 (pharmaceutical compounds).

The optimized SPE-UHPLC-MS/MS has the great advantage of using an eco-friendly solvent (ethanol) for both SPE procedure and UHPLC analysis, according to the recent concerns about green analytical chemistry applied to environmental analyses. Additional advantages presented by the method are the short run time (15 min) and low volume of eluent employed for each analysis, the use of a unique cartridge to extract all the target analytes in a single SPE procedure and the low volume of sample used. To our knowledge, this is the first green methodology to evaluate several micropollutants defined in the European water policy in DW. The SPE-UHPLC-MS/MS method validation was performed according to the international guidelines and the results obtained for selectivity, linearity and range, MDL and MQL, accuracy, recovery and precision were in agreement with these guidelines.

The application of the method to the analysis of tap, fountain and well water samples from different locations of the north of Portugal, showed a widespread occurrence of micropollutants in such matrices, at ng L^{-1} levels. Thirteen compounds were detected in DW samples. The most common micropollutants detected were diclofenac, trimethoprim, warfarin, norfluoxetine, atrazine and simazine. Diclofenac is already included in the first watch list of EU Decision 2015/495 and the pesticides atrazine and simazine are considered PSs by Directive 2013/39/EU.

UV photolysis and ozonation experiments were performed to assess the removal of the target micropollutants, using tap water samples collected from the water supply network of Porto (Portugal) and spiked with the micropollutants. Only eight pharmaceutical compounds were completely removed by the lab-scale experiments, namely tramadol, venlafaxine, atorvastatin and azithromycin by both processes; clopidrogel, carbamazepine and isoproturon by ozonation; and the metabolite norfluoxetine by UV photolysis. The results of this work indicate that future research is needed to promote the complete degradation of this kind of micropollutants, and the by-products that might be formed during these treatments, by using other operating conditions or more efficient technologies.

6. Future work

The SPE-UHPLC-MS/MS analytical method developed and validated in this thesis could be useful in further studies, involving environmental monitorization of the target micropollutants, as well as water treatment options to remove them. Specifically, other tasks should be addressed in the future:

- To extend the SPE-UHPLC-MS/MS for other compounds and possible by-products, especially for micropollutants included in the first watch list of EU Decision 2015/495 and considered PSs by Directive 2013/39/EU;
- To develop a sampling campaign during 1 year for collection and analysis of DW samples, allowing to study the spatial and seasonal occurrence of micropollutants;
- To develop a sampling campaign for collection and analysis of DW samples before and after treatment in DWTP, as well as during the different steps of the treatment, to assess their removal in each stage;
- To study different treatment technologies to remove micropollutants considered as CECs and PSs, using spiked DW; to identify the by-products produced during the application of these processes;
- To establish possible degradation pathways of micropollutants, implementing more powerful analytical techniques.

7. Outputs

The results of this work were disseminated in two conferences [92, 93] and one manuscript is under preparation for submission to a scientific international journal [94].

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Appendix A

A1 - List of priority substances in the field of water policy

Table 8 - Priority substances defined in the Directive 2013/39/EU [7].

Number	CAS number (1)	EU number (2)	Name of priority substance (3)	Identified as priority hazardous substance
(1)	15972-60-8	240-110-8	Alachlor	
(2)	120-12-7	204-371-1	Anthracene	X
(3)	1912-24-9	217-617-8	Atrazine	
(4)	71-43-2	200-753-7	Benzene	
(5)	not applicable	not applicable	Brominated diphenylethers	X (4)
(6)	7440-43-9	231-152-8	Cadmium and its compounds	X
(7)	85535-84-8	287-476-5	Chloroalkanes, C_{10-13}	X
(8)	470-90-6	207-432-0	Chlorfenvinphos	
(9)	2921-88-2	220-864-4	Chlorpyrifos (Chlorpyrifos-ethyl)	
(10)	107-06-2	203-458-1	1,2-dichloroethane	
(11)	75-09-2	200-838-9	Dichloromethane	
(12)	117-81-7	204-211-0	Di(2-ethylhexyl)phthalate (DEHP)	X
(13)	330-54-1	206-354-4	Diuron	
(14)	115-29-7	204-079-4	Endosulfan	X
(15)	206-44-0	205-912-4	Fluoranthene	
(16)	118-74-1	204-273-9	Hexachlorobenzene	X
(17)	87-68-3	201-765-5	Hexachlorobutadiene	X
(18)	608-73-1	210-168-9	Hexachlorocyclohexane	X
(19)	34123-59-6	251-835-4	Isoproturon	
(20)	7439-92-1	231-100-4	Lead and its compounds	
(21)	7439-97-6	231-106-7	Mercury and its compounds	X
(22)	91-20-3	202-049-5	Naphthalene	
(23)	7440-02-0	231-111-4	Nickel and its compounds	

Table 8 - Continued.

Number	CAS number (1)	EU number (2)	Name of priority substance (3)	Identified as priority hazardous substance
(24)	not applicable	not applicable	Nonylphenols	X (5)
(25)	not applicable	not applicable	Octylphenols (6)	
(26)	608-93-5	210-172-0	Pentachlorobenzene	X
(27)	87-86-5	201-778-6	Pentachlorophenol	
(28)	not applicable	not applicable	Polyaromatic hydrocarbons (PAH) (7)	X
(29)	122-34-9	204-535-2	Simazine	
(30)	not applicable	not applicable	Tributyltin compounds	X (8)
(31)	12002-48-1	234-413-4	Trichlorobenzenes	
(32)	67-66-3	200-663-8	Trichloromethane (chloroform)	
(33)	1582-09-8	216-428-8	Trifluralin	X
(34)	115-32-2	204-082-0	Dicofol	X
(35)	1763-23-1	217-179-8	Perfluorooctane sulfonic acid and its derivatives (PFOS)	X
(36)	124495-18-7	not applicable	Quinoxyfen	X
(37)	not applicable	not applicable	Dioxins and dioxin-like compounds	X (9)
(38)	74070-46-5	277-704-1	Aclonifen	
(39)	42576-02-3	255-894-7	Bifenox	
(40)	28159-98-0	248-872-3	Cybutryne	
(41)	52315-07-8	257-842-9	Cypermethrin (10)	
(42)	62-73-7	200-547-7	Dichlorvos	
(43)	not applicable	not applicable	Hexabromocyclododecanes (HBCDD)	X (11)
(44)	76-44-8/ 1024-57-3	200-962-3/ 213-831-0	Heptachlor and heptachlor epoxide	X
(45)	886-50-0	212-950-5	Terbutryn	

(1) CAS: Chemical Abstracts Service.

(2) EU-number: European Inventory of Existing Commercial Substances (EINECS) or European List of Notified Chemical Substances (ELINCS).

(3) Where groups of substances have been selected, unless explicitly noted, typical individual representatives are defined in the context of the setting of environmental quality standards.

(4) Only Tetra, Penta, Hexa and Heptabromodiphenylether (CAS -numbers 40088-47-9, 32534-81-9, 36483-60-0, 68928-80-3, respectively).

(5) Nonylphenol (CAS 25154-52-3, EU 246-672-0) including isomers 4-nonylphenol (CAS 104-40-5, EU 203-199-4) and 4-nonylphenol (branched) (CAS 84852-15-3, EU 284-325-5).

(6) Octylphenol (CAS 1806-26-4, EU 217-302-5) including isomer 4-(1,1',3,3'-tetramethylbutyl)-phenol (CAS 140-66-9, EU 205-426-2).

([7]) Including benzo(a)pyrene (CAS 50-32-8, EU 200-028-5), benzo(b)fluoranthene (CAS 205-99-2, EU 205-911-9), benzo(g,h,i)perylene (CAS 191-24-2, EU 205-883-8), benzo(k)fluoranthene (CAS 207-08-9, EU 205-916-6), indeno(1,2,3-cd)pyrene (CAS 193-39-5, EU 205-893-2) and excluding anthracene, fluoranthene and naphthalene, which are listed separately.

([8]) Including tributyltin-cation (CAS 36643-28-4).

([9]) This refers to the following compounds:

7 polychlorinated dibenzo-p -dioxins (PCDDs): 2,3,7,8-T4CDD (CAS 1746-01-6), 1,2,3,7,8-P5CDD (CAS 40321-76-4), 1,2,3,4,7,8-H6CDD (CAS 39227-28-6), 1,2,3,6,7,8-H6CDD (CAS 57653-85-7), 1,2,3,7,8,9-H6CDD (CAS 19408-74-3), 1,2,3,4,6,7,8-H7CDD (CAS 35822-46-9), 1,2,3,4,6,7,8,9-O8CDD (CAS 3268-87-9)

10 polychlorinated dibenzofurans (PCDFs): 2,3,7,8-T4CDF (CAS 51207-31-9), 1,2,3,7,8-P5CDF (CAS 57117-41-6), 2,3,4,7,8-P5CDF (CAS 57117-31-4), 1,2,3,4,7,8-H6CDF (CAS 70648-26-9), 1,2,3,6,7,8-H6CDF (CAS 57117-44-9), 1,2,3,7,8,9-H6CDF (CAS 72918-21-9), 2,3,4,6,7,8-H6CDF (CAS 60851-34-5), 1,2,3,4,6,7,8-H7CDF (CAS 67562-39-4), 1,2,3,4,7,8,9-H7CDF (CAS 55673-89-7), 1,2,3,4,6,7,8,9-O8CDF (CAS 39001-02-0)

12 dioxin-like polychlorinated biphenyls (PCB-DL): 3,3',4,4'-T4CB (PCB 77, CAS 32598-13-3), 3,3',4',5-T4CB (PCB 81, CAS 70362-50-4), 2,3,3',4,4'-P5CB (PCB 105, CAS 32598-14-4), 2,3,4,4',5-P5CB (PCB 114, CAS 74472-37-0), 2,3',4,4',5-P5CB (PCB 118, CAS 31508-00-6), 2,3',4,4',5'-P5CB (PCB 123, CAS 65510-44-3), 3,3',4,4',5-P5CB (PCB 126, CAS 57465-28-8), 2,3,3',4,4',5-H6CB (PCB 156, CAS 38380-08-4), 2,3,3',4,4',5'-H6CB (PCB 157, CAS 69782-90-7), 2,3',4,4',5,5'-H6CB (PCB 167, CAS 52663-72-6), 3,3',4,4',5,5'-H6CB (PCB 169, CAS 32774-16-6), 2,3,3',4,4',5,5'-H7CB (PCB 189, CAS 39635-31-9).

([10]) CAS 52315-07-8 refers to an isomer mixture of cypermethrin, alpha-cypermethrin (CAS 67375-30-8), beta-cypermethrin (CAS 65731-84-2), theta-cypermethrin (CAS 71697-59-1) and zeta-cypermethrin (52315-07-8).

([11]) This refers to 1,3,5,7,9,11-Hexabromocyclododecane (CAS 25637-99-4), 1,2,5,6,9,10-Hexabromocyclododecane (CAS 3194-55-6), α-Hexabromocyclododecane (CAS 134237-50-6), ß-Hexabromocyclododecane (CAS 134237-51-7) and γ- Hexabromocyclododecane (CAS 134237-52-8).

A2 - Watch list of substances for Union-wide monitoring in the field of water policy

Table 9 - Watch list of substances for Union-wide monitoring in the field of water policy defined in the COMMISSION IMPLEMENTING DECISION (EU) 2015/495 of 20 March 2015 [8].

Name of substance/group of substances	CAS number (1)	EU number (2)	Indicative analytical method (3) (4) (5)	Maximum acceptable method detection limit (ng/l)
17-Alpha-ethinylestradiol (EE2)	57-63-6	200-342-2	Large-volume SPE — LC-MS-MS	0,035
17-Beta-estradiol (E2), Estrone (E1)	50-28-2, 53-16-7	200-023-8	SPE — LC-MS-MS	0,4
Diclofenac	15307-86-5	239-348-5	SPE — LC-MS-MS	10
2,6-Ditert-butyl-4-methylphenol	128-37-0	204-881-4	SPE — GC-MS	3 160
2-Ethylhexyl 4-methoxycinnamate	5466-77-3	226-775-7	SPE — LC-MS-MS or GC-MS	6 000
Macrolide antibiotics (6)			SPE — LC-MS-MS	90
Methiocarb	2032-65-7	217-991-2	SPE — LC-MS-MS or GC-MS	10
Neonicotinoids (7)			SPE — LC-MS-MS	9
Oxadiazon	19666-30-9	243-215-7	LLE/SPE — GC-MS	88
Tri-allate	2303-17-5	218-962-7	LLE/SPE — GC-MS or LC-MS-MS	670

(1) Chemical Abstracts Service.

(2) European Union number — not available for all substances.

(3) To ensure comparability of results from different Member States, all substances shall be monitored in whole water samples.

(4) Extraction methods: LLE — liquid-liquid extraction, SPE — solid-phase extraction.

Analytical methods: GC-MS — Gas chromatography-mass spectrometry,

(5) LC-MS-MS — Liquid chromatography (tandem) triple quadrupole mass spectrometry.

For monitoring 2-Ethylhexyl 4-methoxycinnamate in suspended particulate matter (SPM) or in sediment (size < 63 µm), the following analytical method is indicated: SLE (solid liquid extraction) — GC-MS, with a maximum detection limit of 0,2 mg/kg.

(6) Erythromycin (CAS number 114-07-8, EU number 204-040-1), Clarithromycin (CAS number 81103-11-9), Azithromycin (CAS number 83905-01-5, EU number 617-500-5).

(7) Imidacloprid (CAS number 105827-78-9/138261-41-3, EU number 428-040-8), Thiacloprid (CAS number 111988-49-9), Thiamethoxam (CAS number 153719-23-4, EU number 428-650-4), Clothianidin (CAS number 210880-92-5, EU number 433-460-1), Acetamiprid (CAS number 135410-20-7/160430-64-8).

Appendix B: Mobile phase

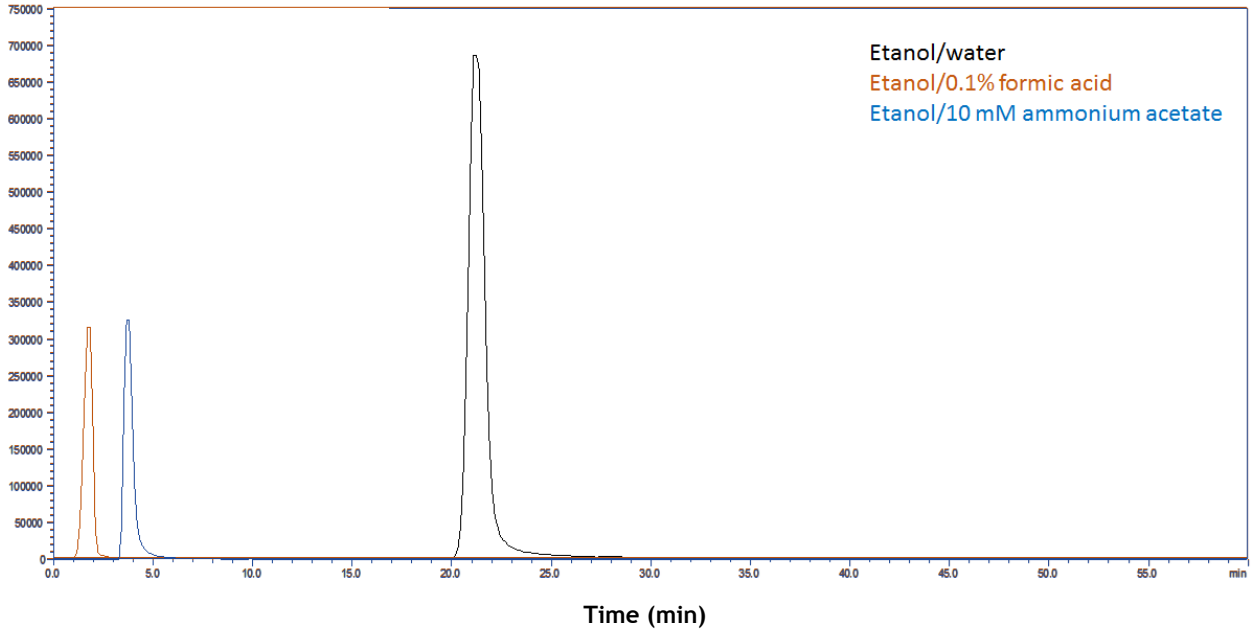

Figure 18 - Chromatograms of fluoxetine obtained with different mobile phases: ethanol/ultrapure water; ethanol/ammonium acetate; ethanol/formic acid.

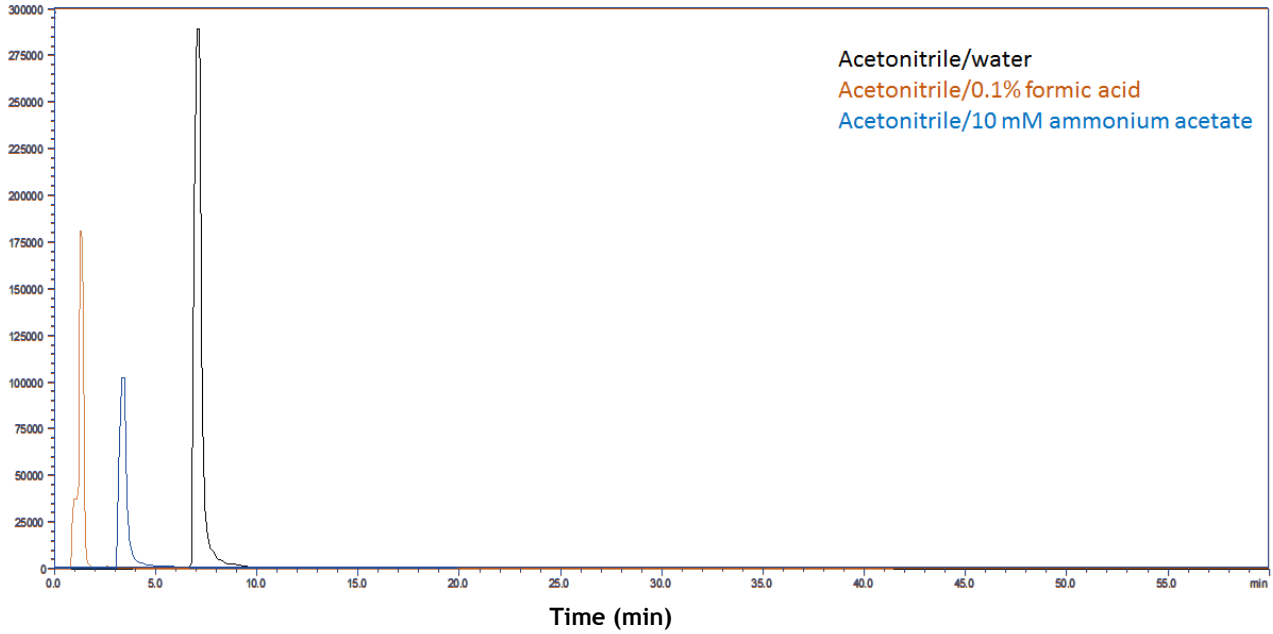

Figure 19 - Chromatograms of fluoxetine obtained with different mobile phases: acetonitrile/ultrapure water; acetonitrile/ammonium acetate; acetonitrile/formic acid.

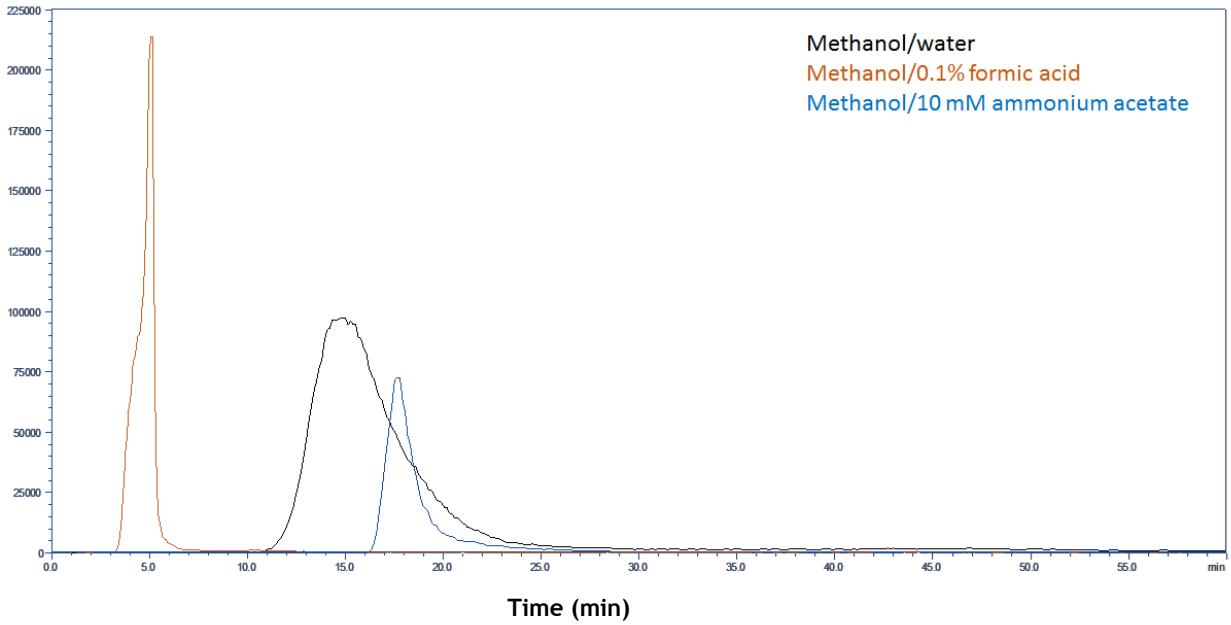

Figure 20 - Chromatograms of fluoxetine obtained with different mobile phases: methanol/ultrapure water; methanol/ammonium acetate; methanol/formic acid.

Appendix C: MS parameters

- **Desolvation temperature**

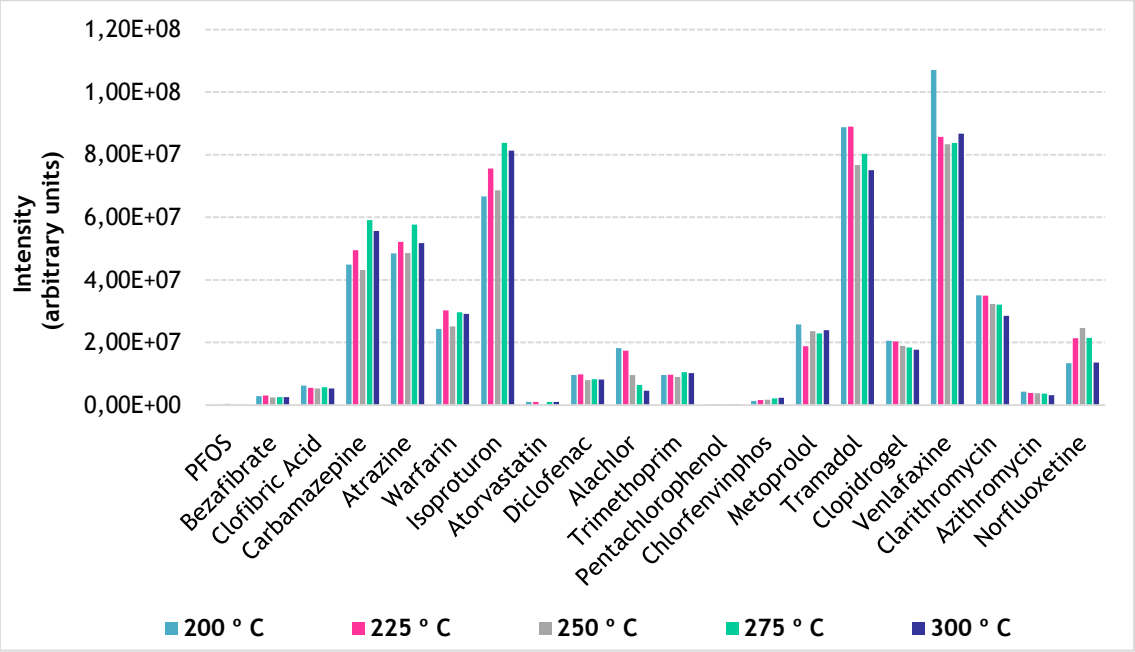

Figure 21 - Results obtained for target micropollutants with different desolvation temperature values: 200, 225, 250, 275, and 300 ° C.

- **Source temperature**

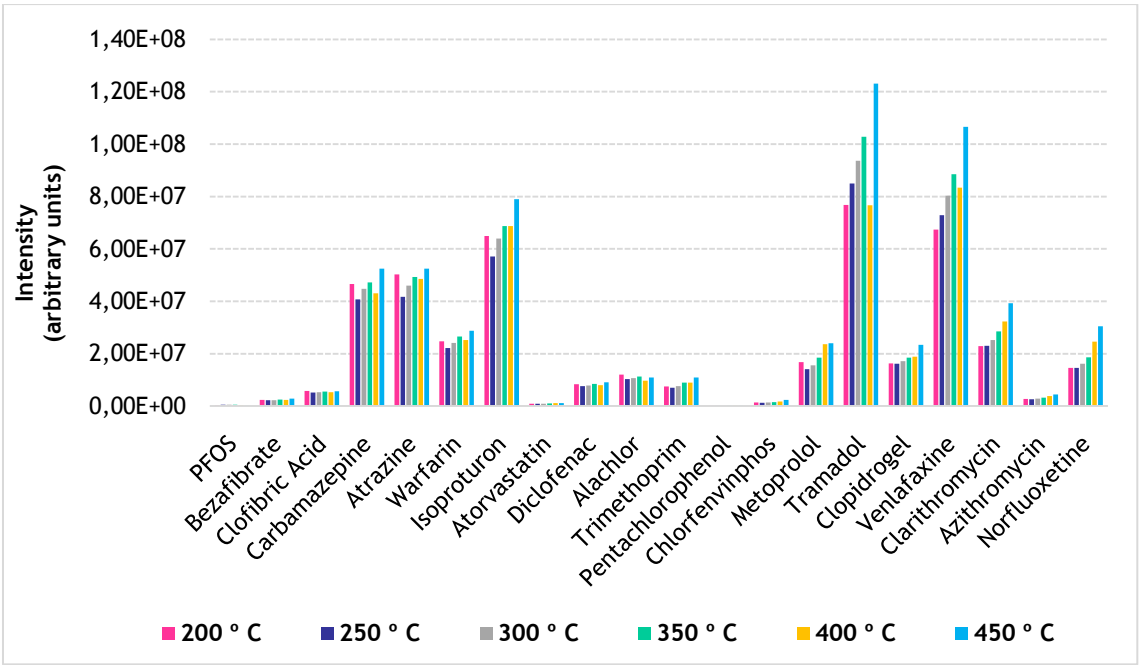

Figure 22 - Results obtained for target micropollutants with different source temperature values: 200, 250, 300, 350, 400 and 450 ° C.

- **Nebulizing gas flow**

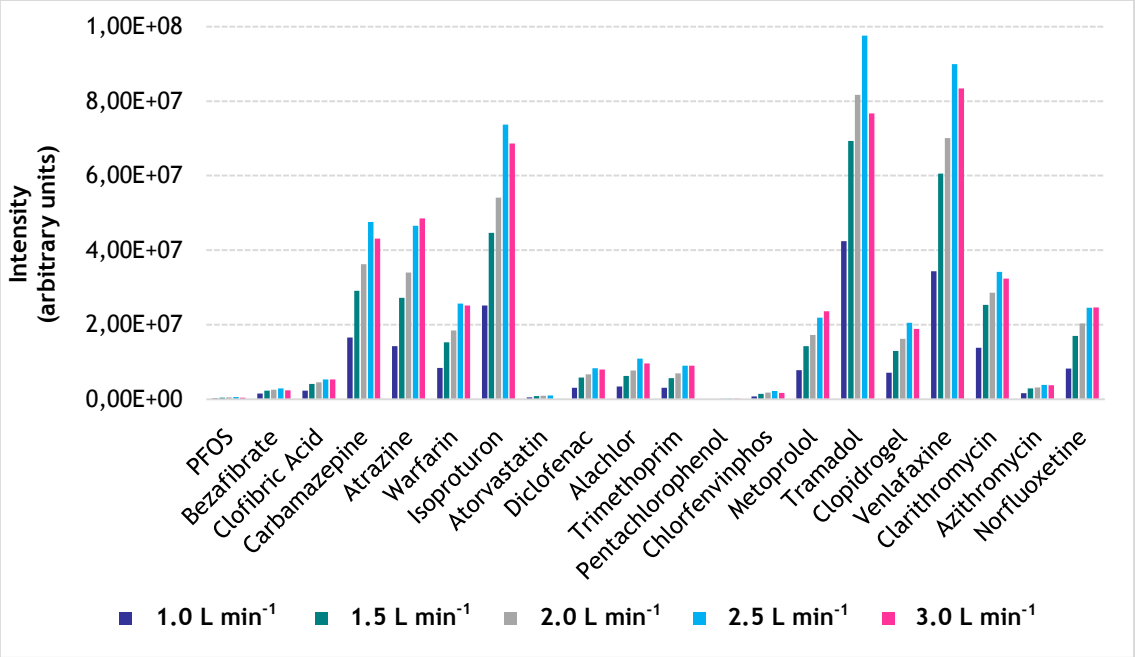

Figure 23 - Results obtained for target micropollutants with different nebulizing gas flow values: 1.0, 1.5, 2.0, 2.5 and 3.0 L min^{-1}.

- **Drying gas flow**

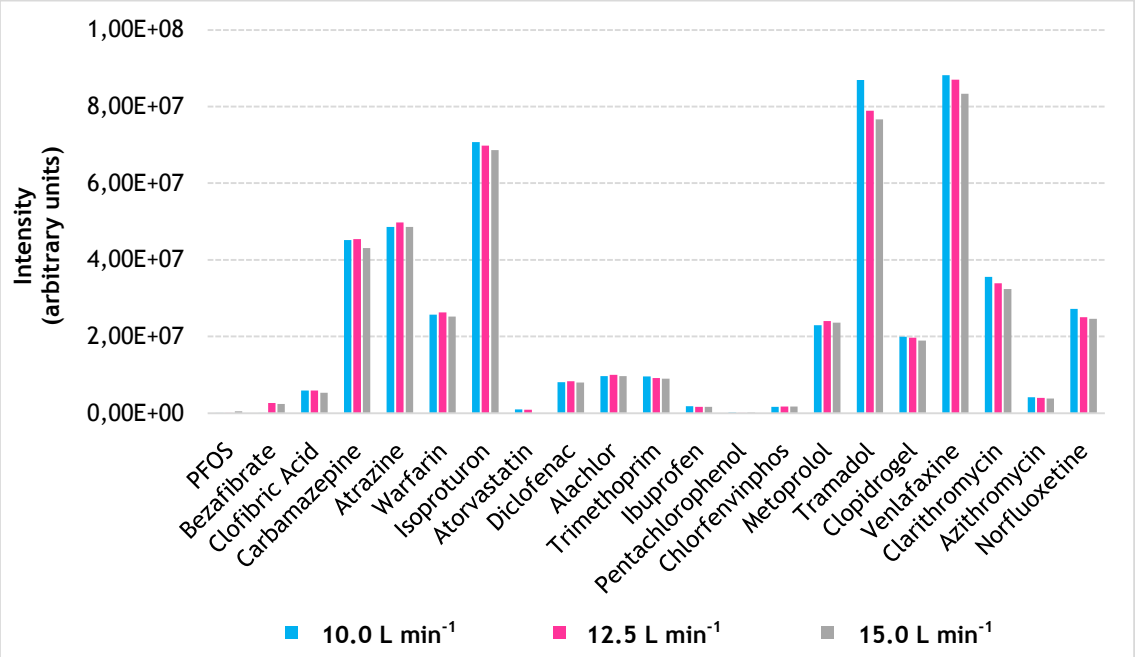

Figure 24 - Results obtained for target micropollutants with different drying gas flow values: 10.0, 12.5 and 15.0 L min^{-1}.

- **Capillary voltage**

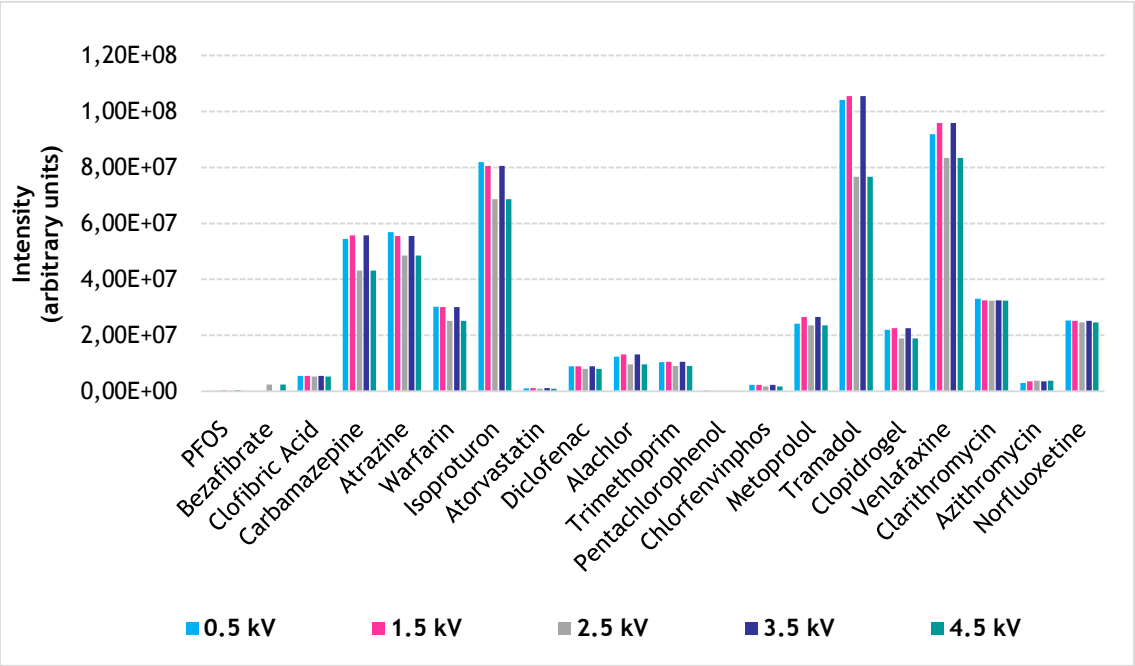

Figure 25 - Results obtained for target micropollutants with different capillary voltage values: 0.5, 1.5, 2.5, 3.5 and 4.5 kV.